QUANTITATIVE

NON-TARGETED SCREENING

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Kruvelab.com

We do not describe the world we see, we see the world we can describe.

~ Rene Descartes

AZ QUOTES

To monitor the health of cities' residents, look no further than their sewers
C&EN 96 (2018)

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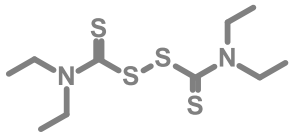
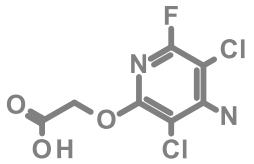
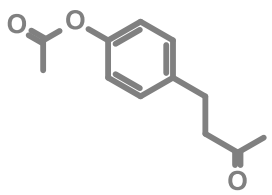
BACKGROUND

OF NON-TARGETED SCREENING

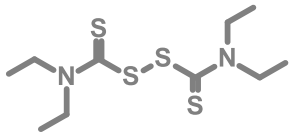
FROM TARGETED TO NON-TARGETED

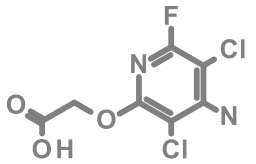


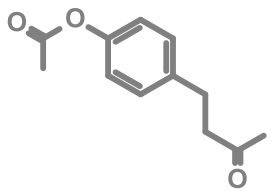


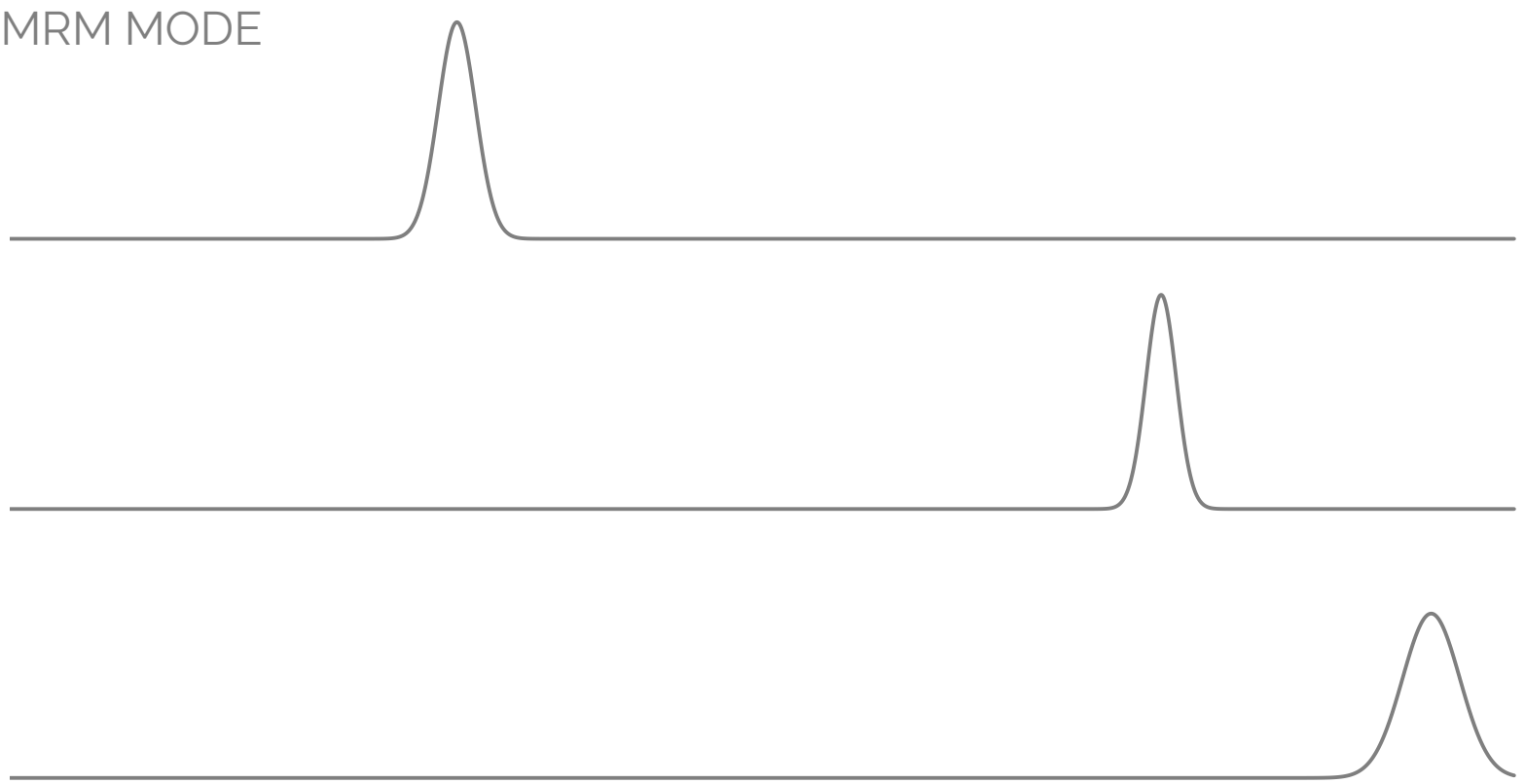


FROM TARGETED TO NON-TARGETED









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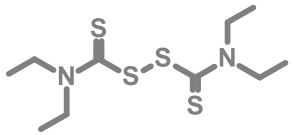
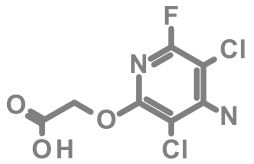
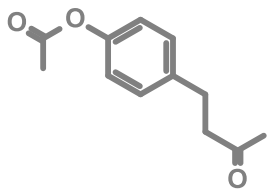
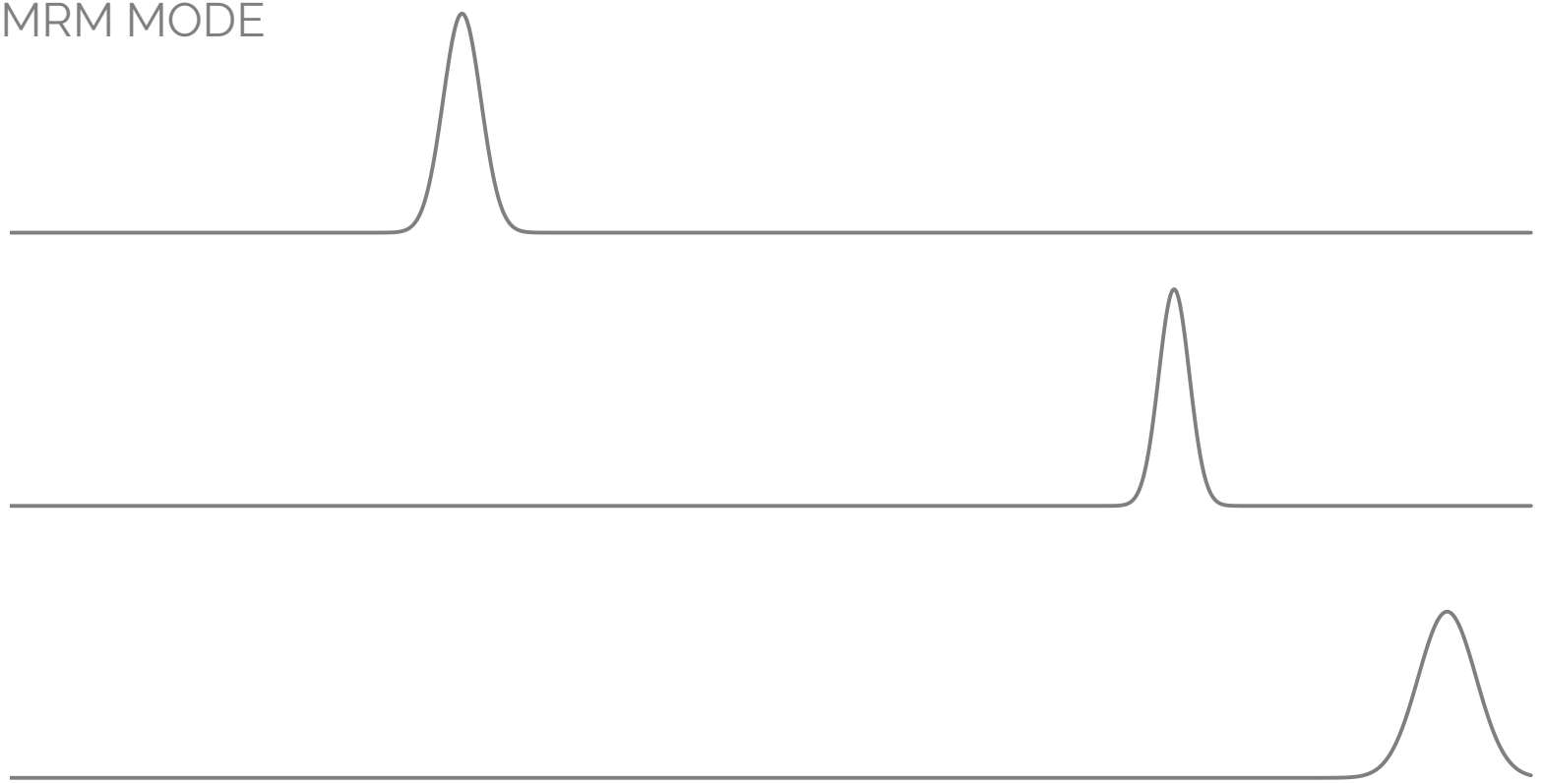




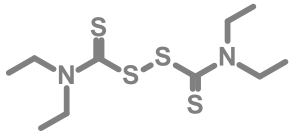


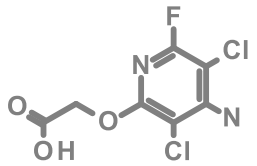
NO TARGETED LIST

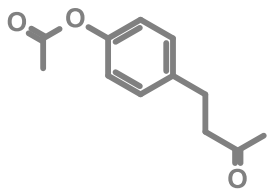
MRM MODE



FROM TARGETED TO NON-TARGETED







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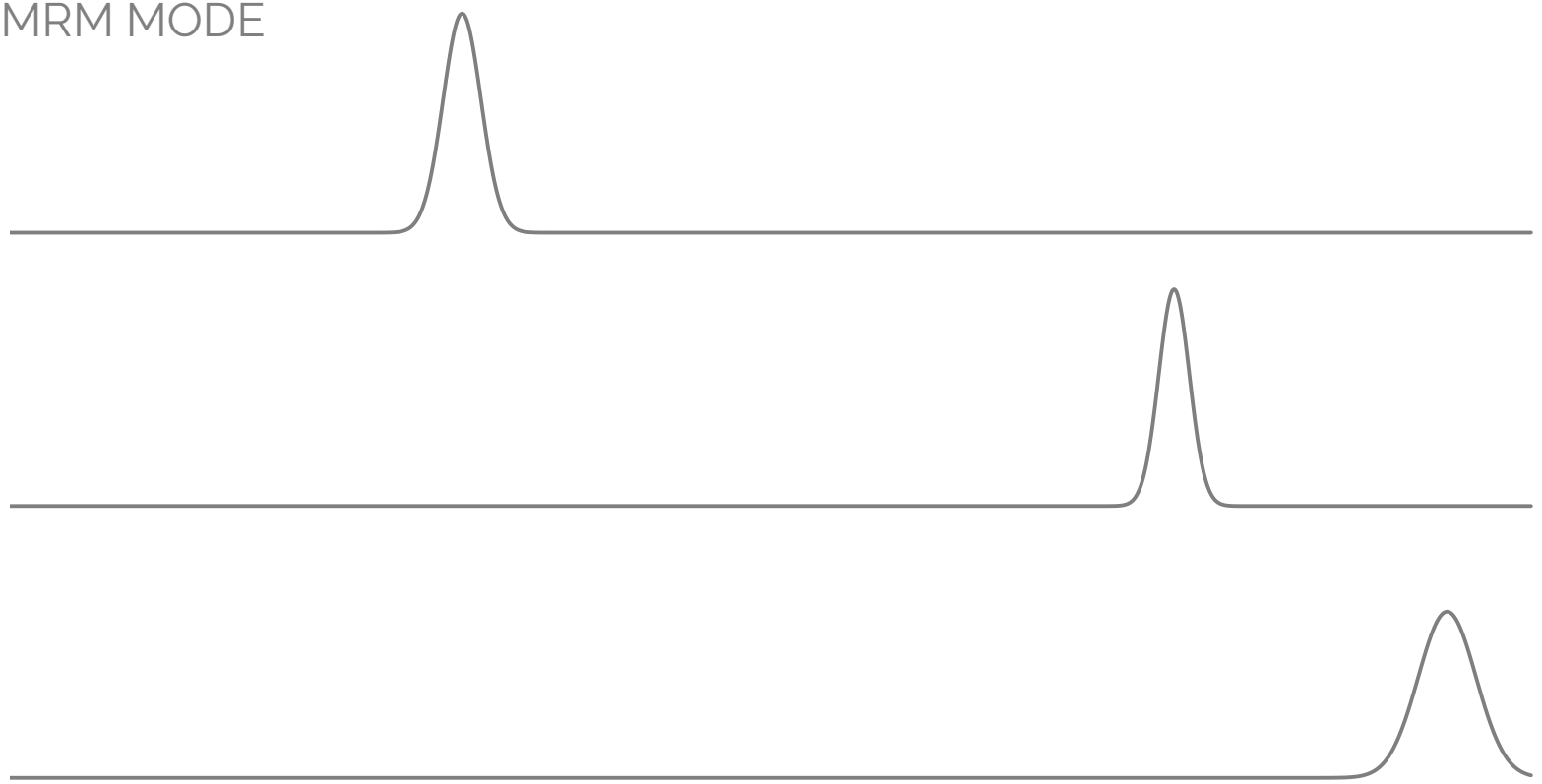
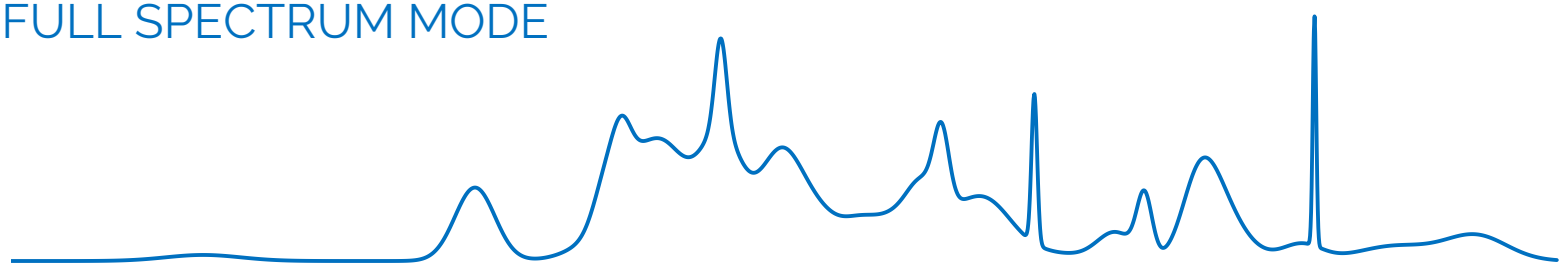


NO TARGETED LIST

FULL SPECTRUM MODE



SCREENING OF SURFACE WATER

IN COLLABORATION WITH KWR

SAMPLES

SURFACE WATER FROM THE NETHERLANDS

28 different locations

FULL SPECTRUM MODE

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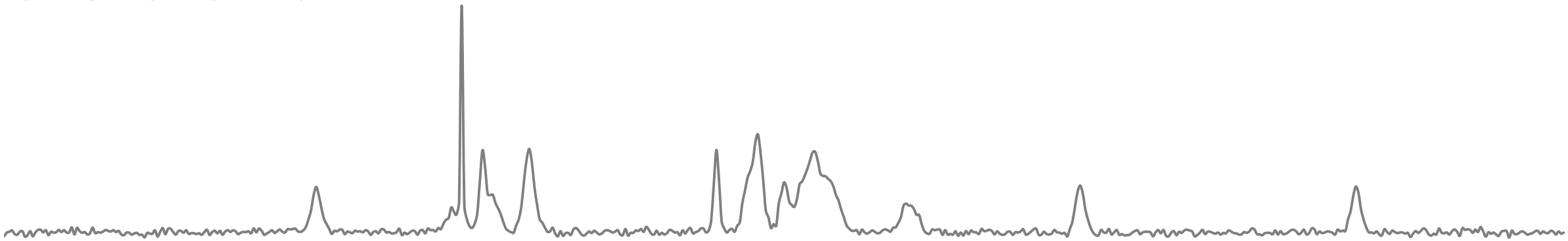
SAMPLES

SURFACE WATER FROM THE NETHERLANDS

28 different locations & 119 different peaks

FULL SPECTRUM MODE



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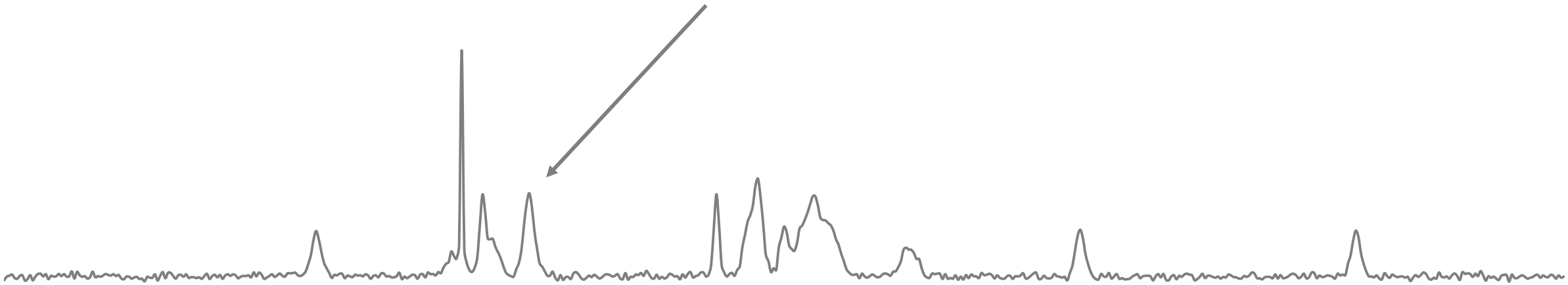
IDENTIFICATION

t_R = 10.1 min



IDENTIFICATION

t_R = 10.1 min

133.0640 | Level 5: Exact mass of interest | MS

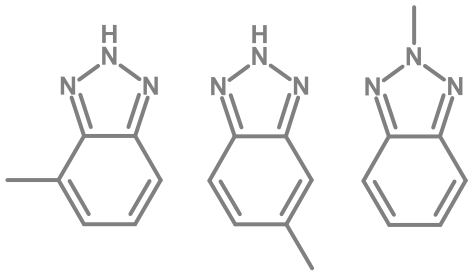
IDENTIFICATION

t_R = 10.1 min

$C_7H_7N_3$	Level 4: Unequivocal molecular formula	MS isotope/adduct
133.0640	Level 5: Exact mass of interest	MS

IDENTIFICATION

t_R = 10.1 min



$C_7H_7N_3$

133.0640

Level 3: Tentative candidate(s) structure, substituent, class	MS, MS^2, Exp. data
Level 4: Unequivocal molecular formula	MS isotope/adduct
Level 5: Exact mass of interest	MS

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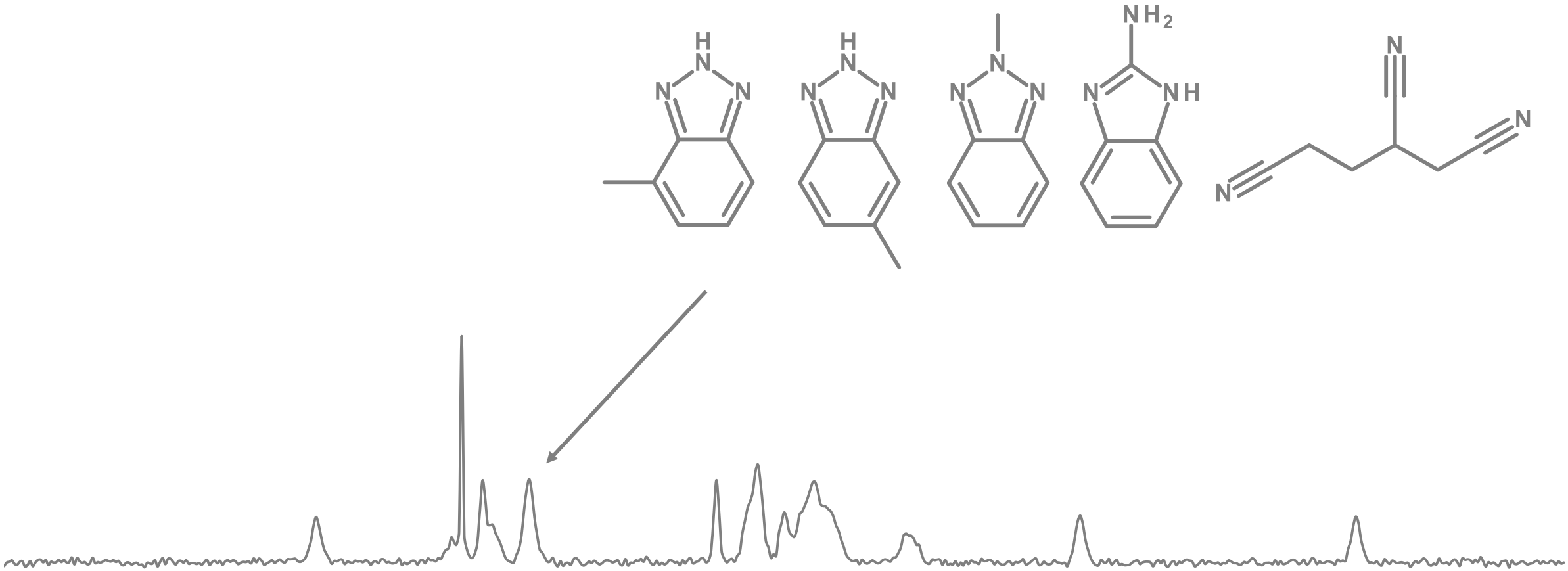
IDENTIFICATION

t_R = 10.1 min

5 possible structures



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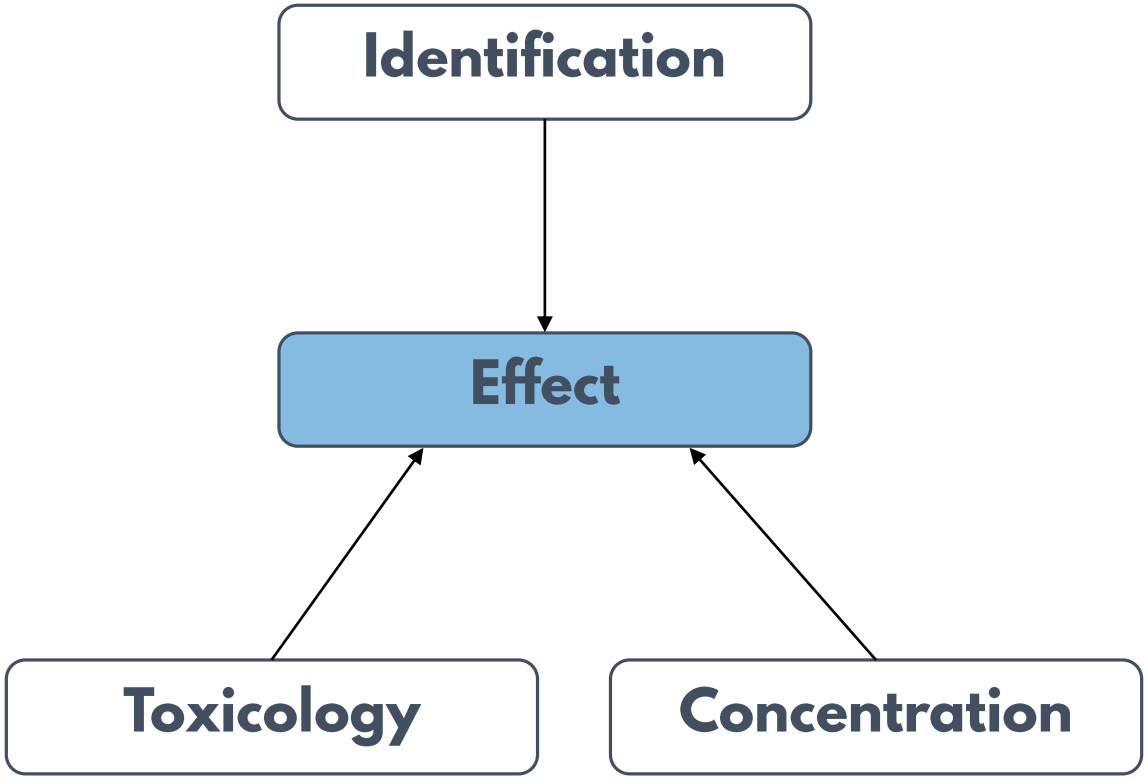
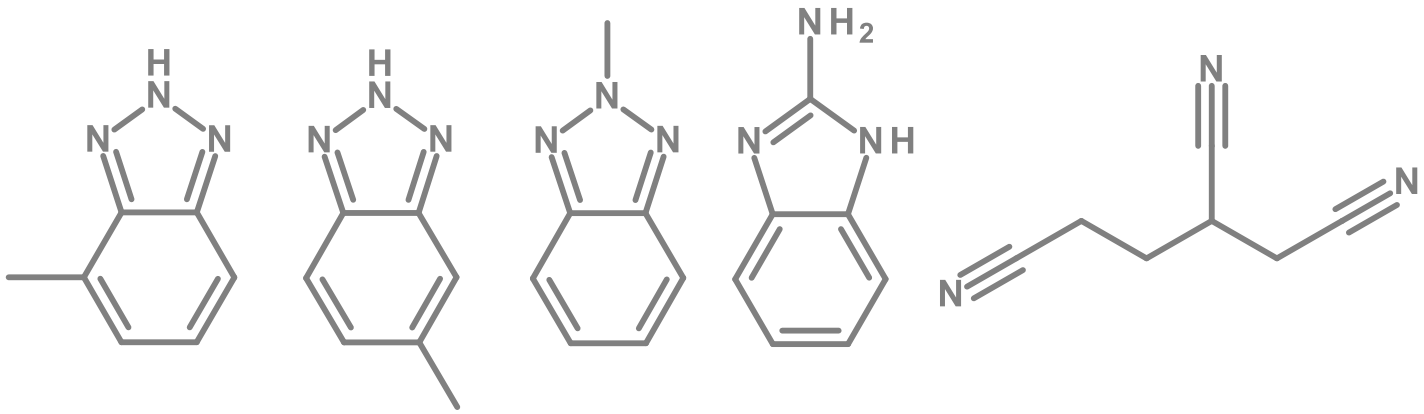
EFFECT DRIVEN INTERPRETATION



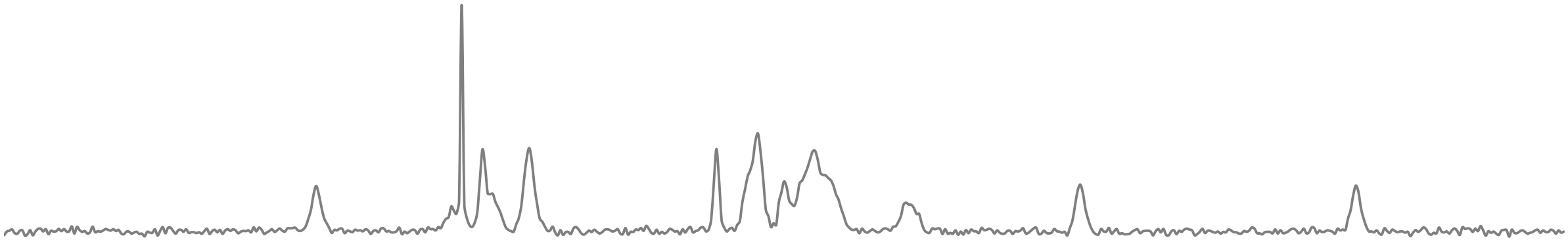
PRIORITIZATION

BASED ON TOXICITY

ToxCast toxicity tests



AC_{50} (μM)	NA	**0.27**	NA	**12.82**	NA



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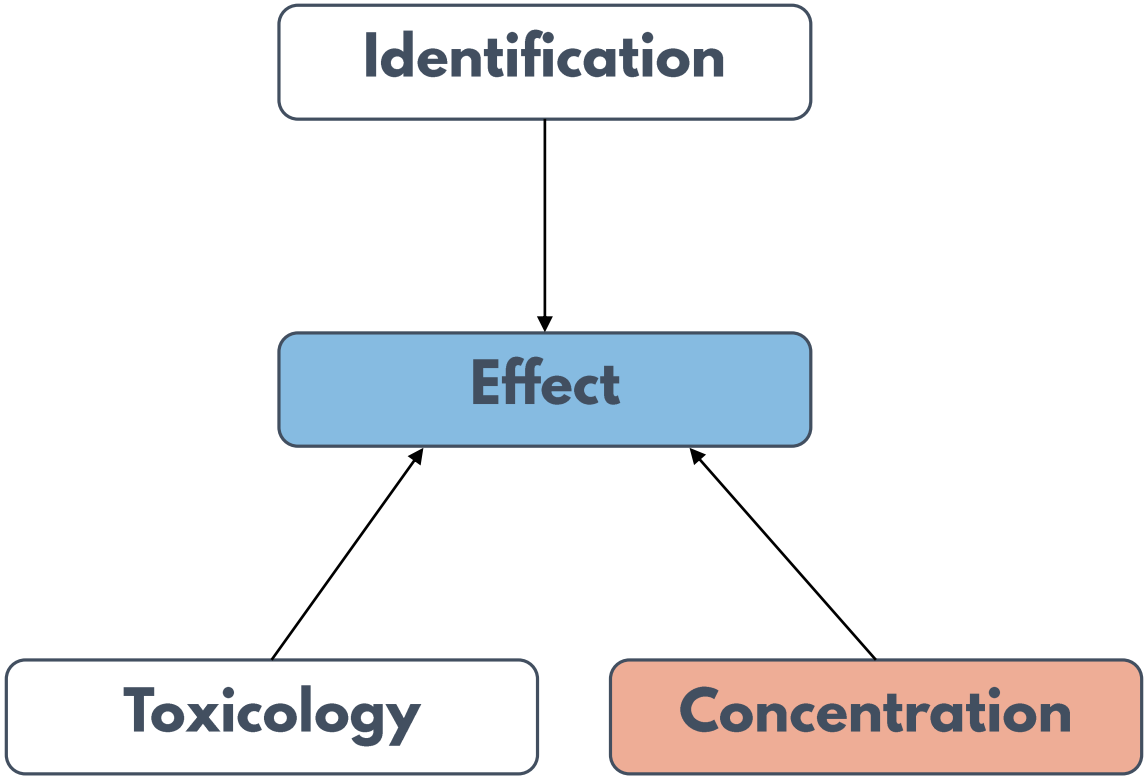
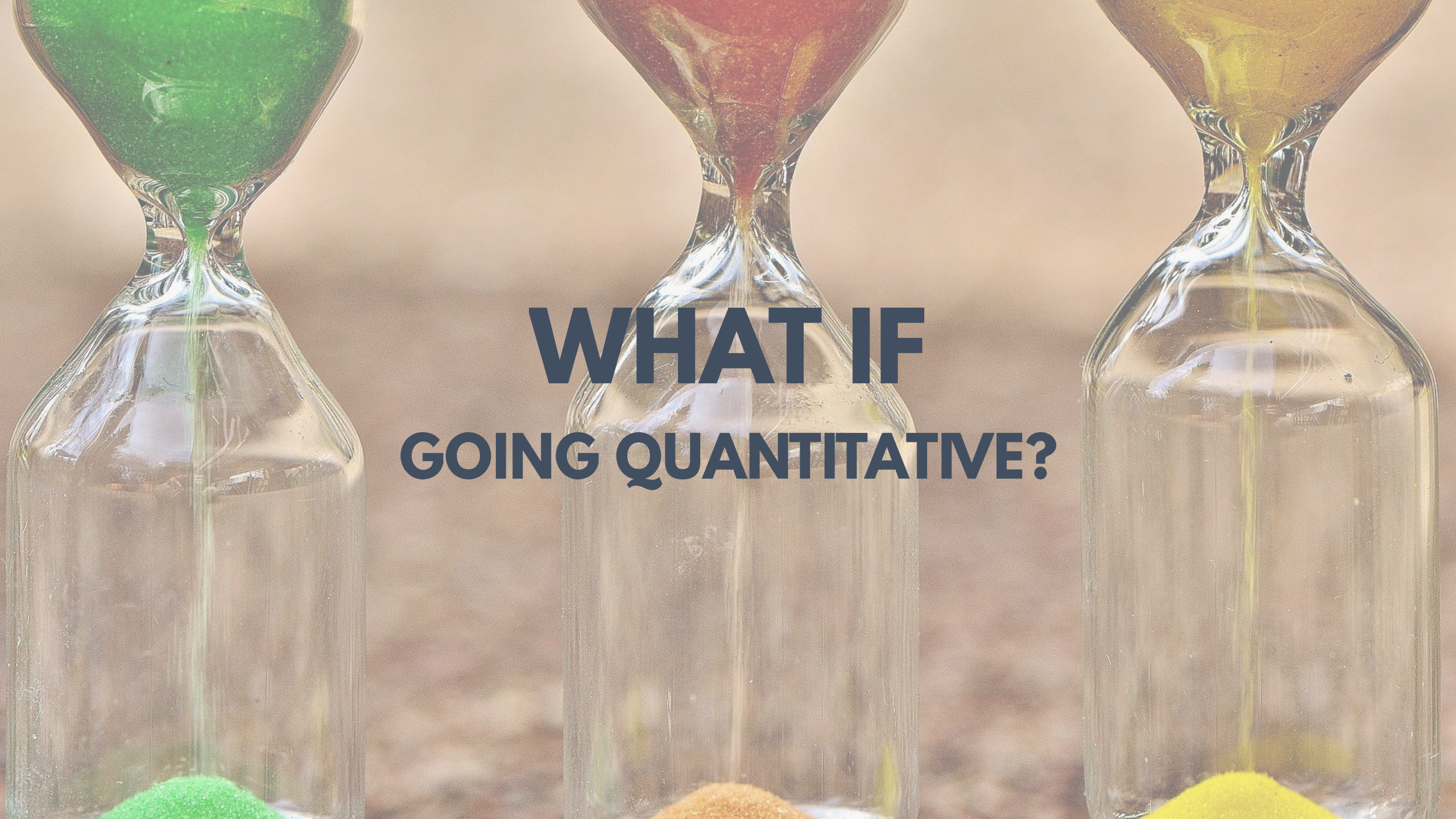
EFFECT DRIVEN INTERPRETATION





WHAT IF

GOING QUANTITATIVE?

QUALITATIVE

stop after identification

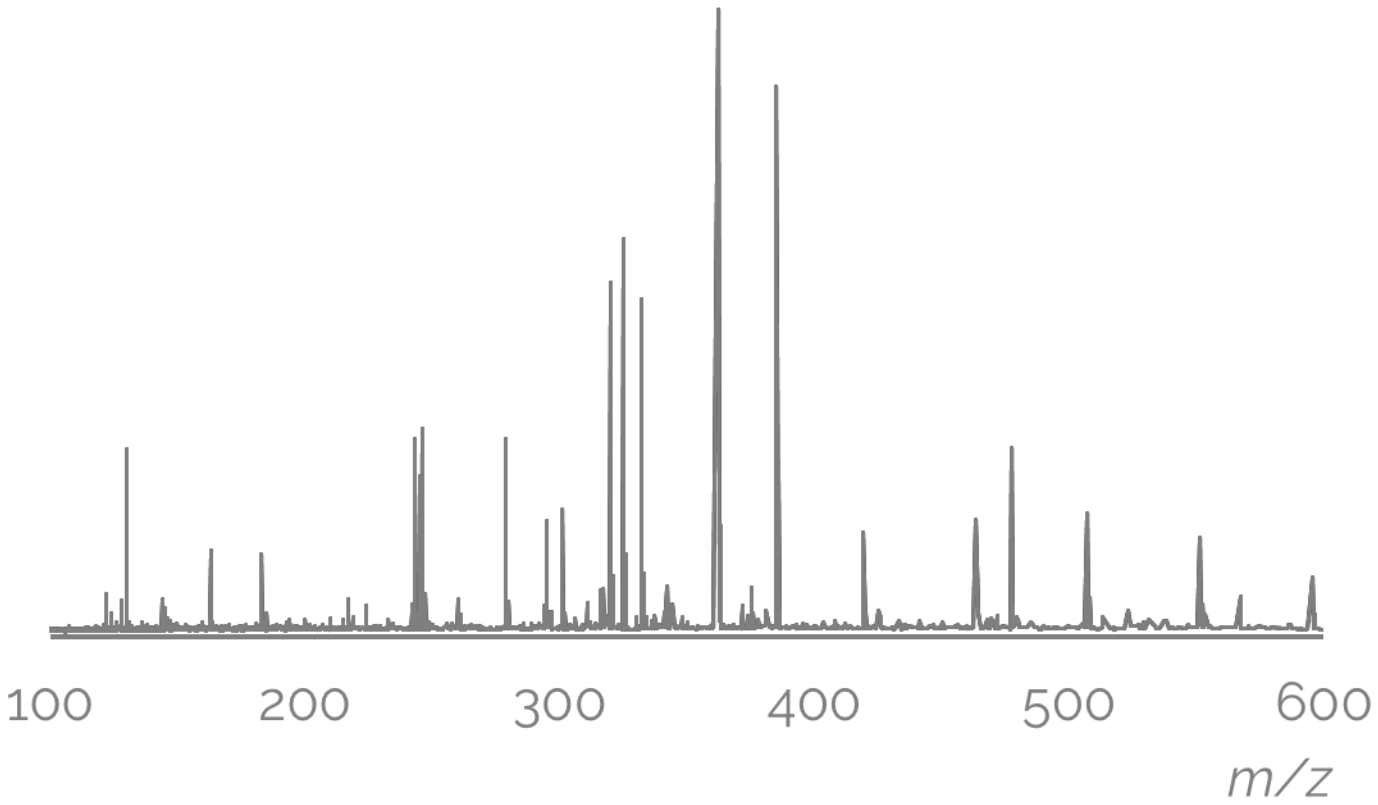
QUANTITATIVE

purchase or synthesize the standard substances

QUANTIFICATION IS COMPLICATED

ESI/HRMS NON-TARGETED ANALYSIS

reveals hundreds of compounds



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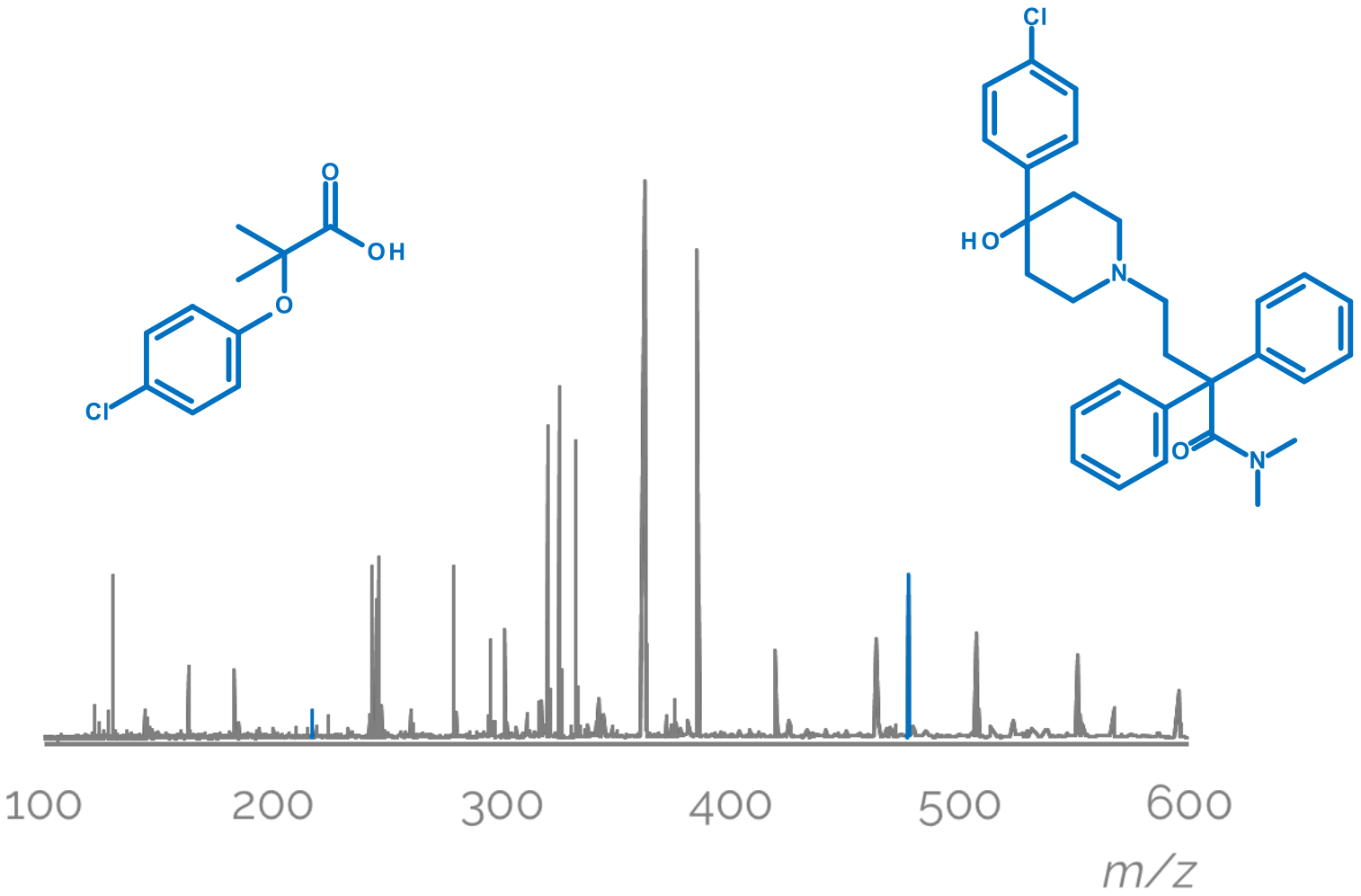
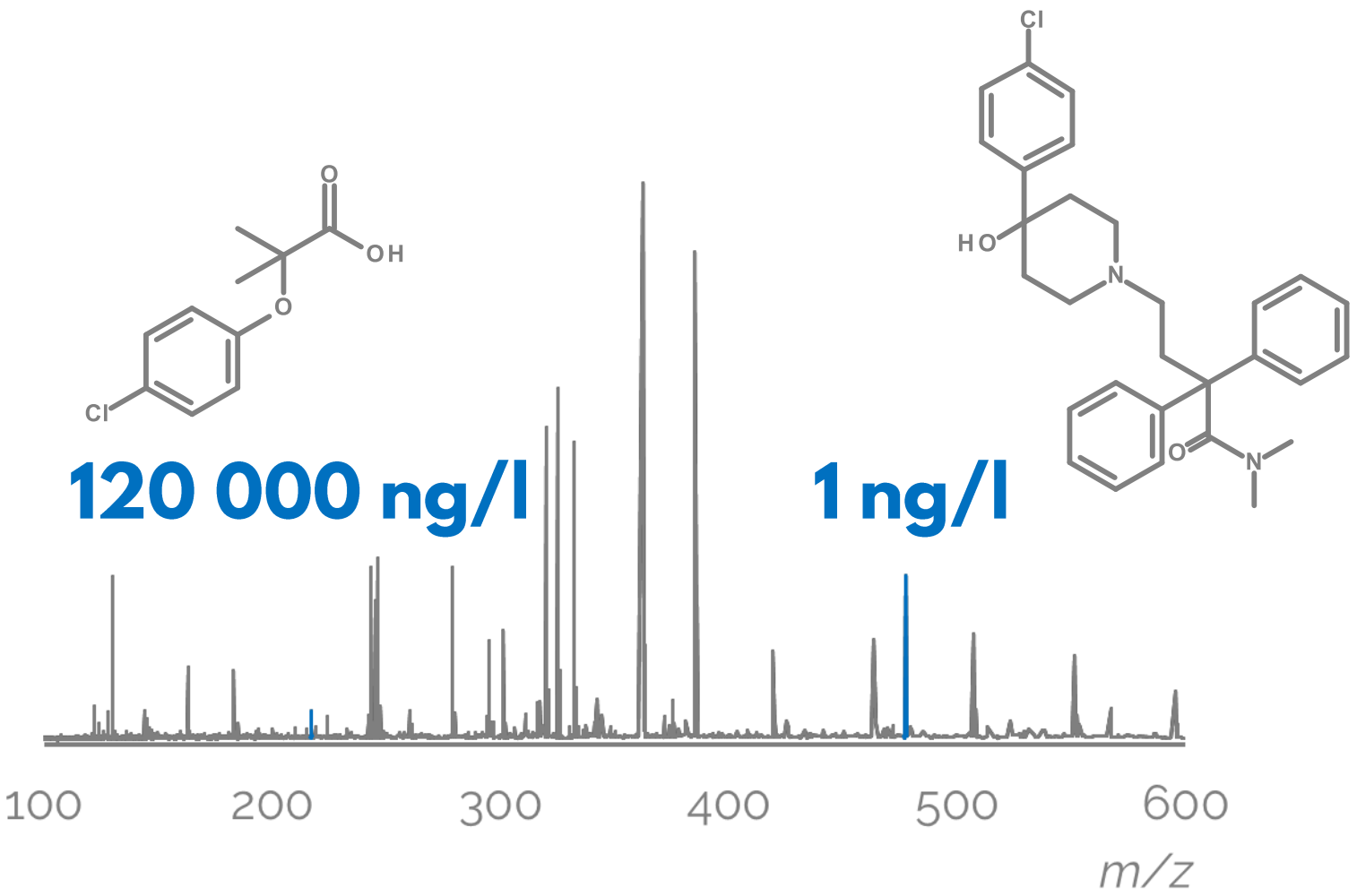
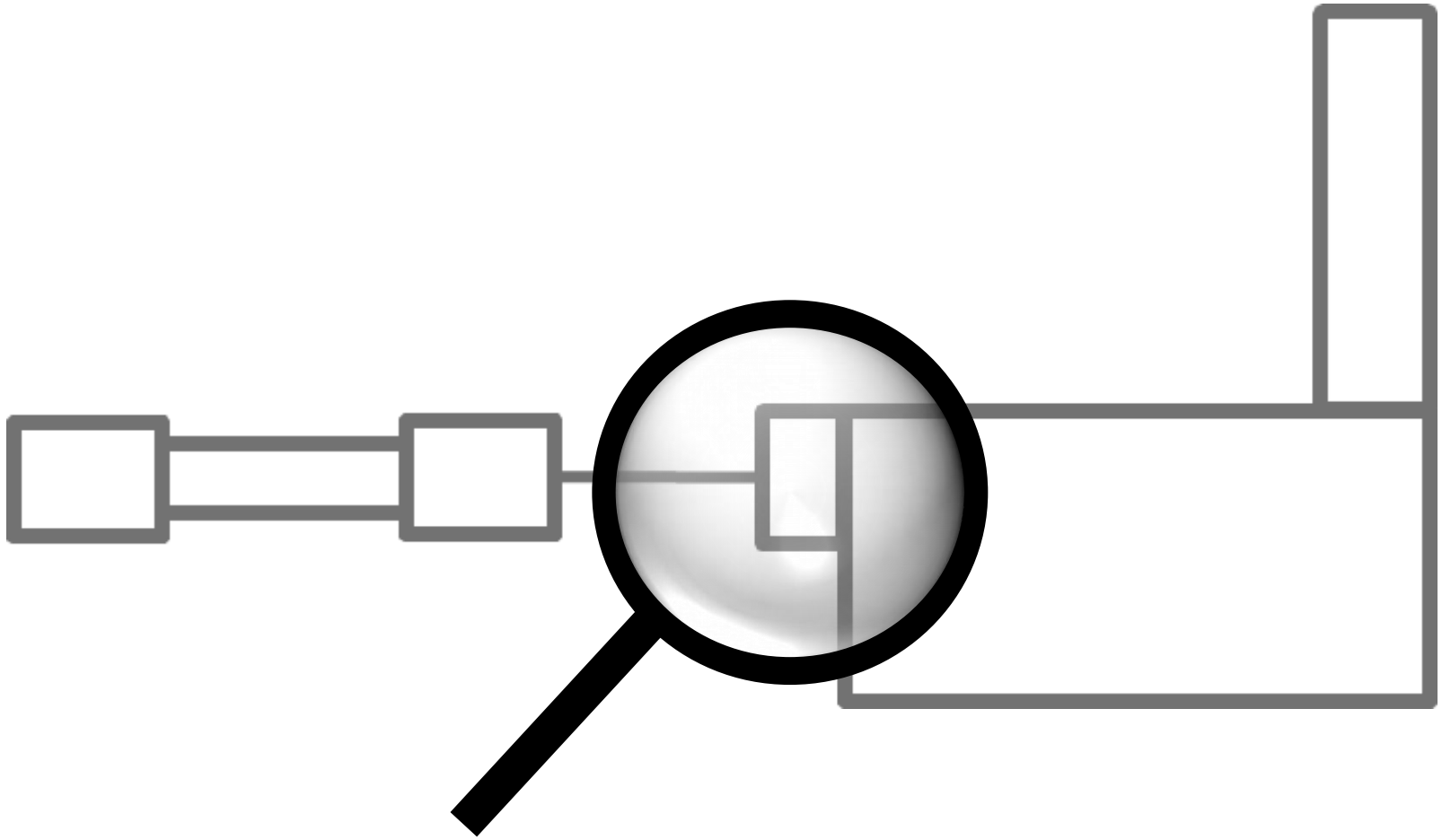


SO WHAT IS THE CONCENTRATION?



ELECTROSPRAY IONIZATION PROCESS



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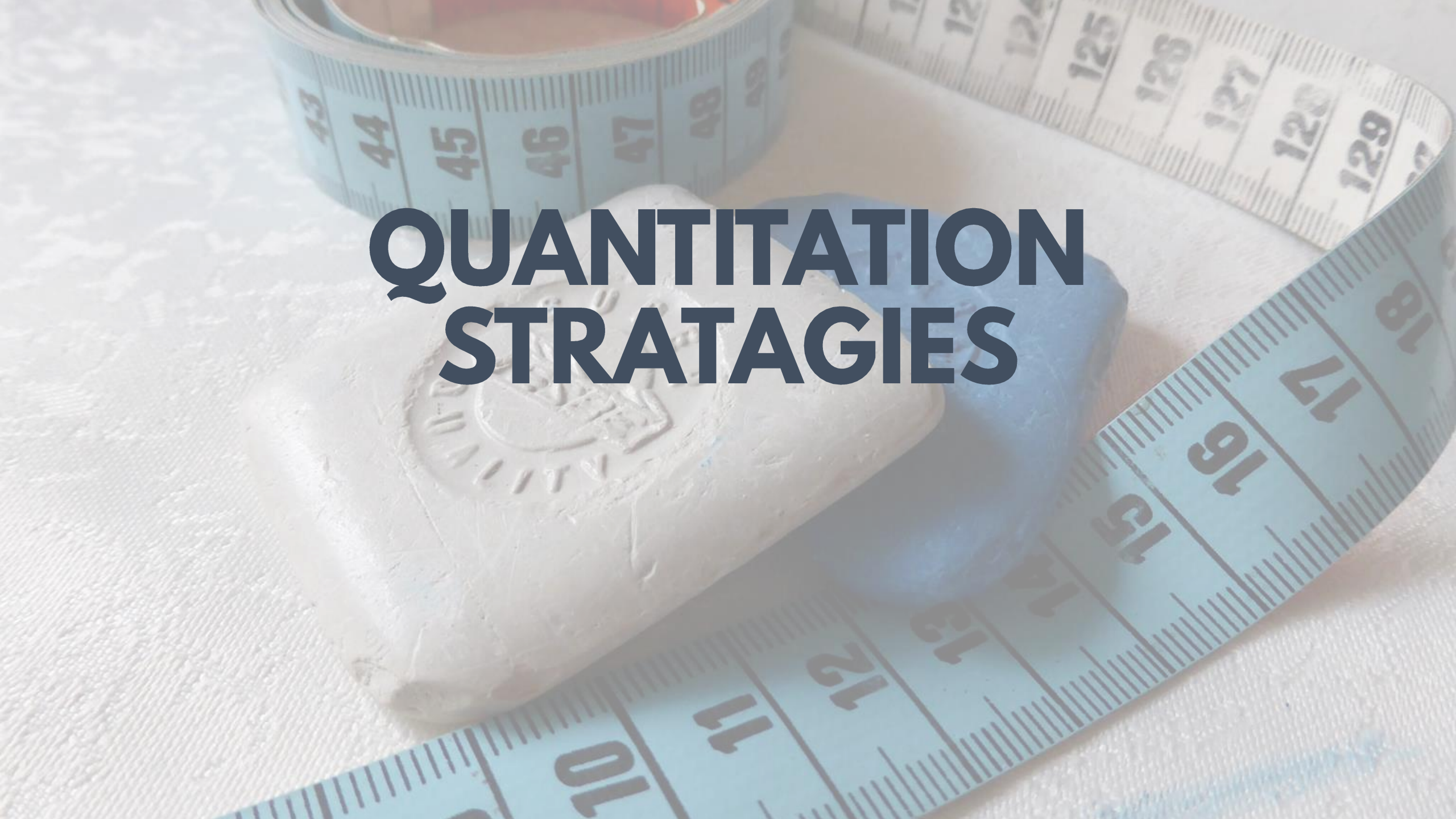
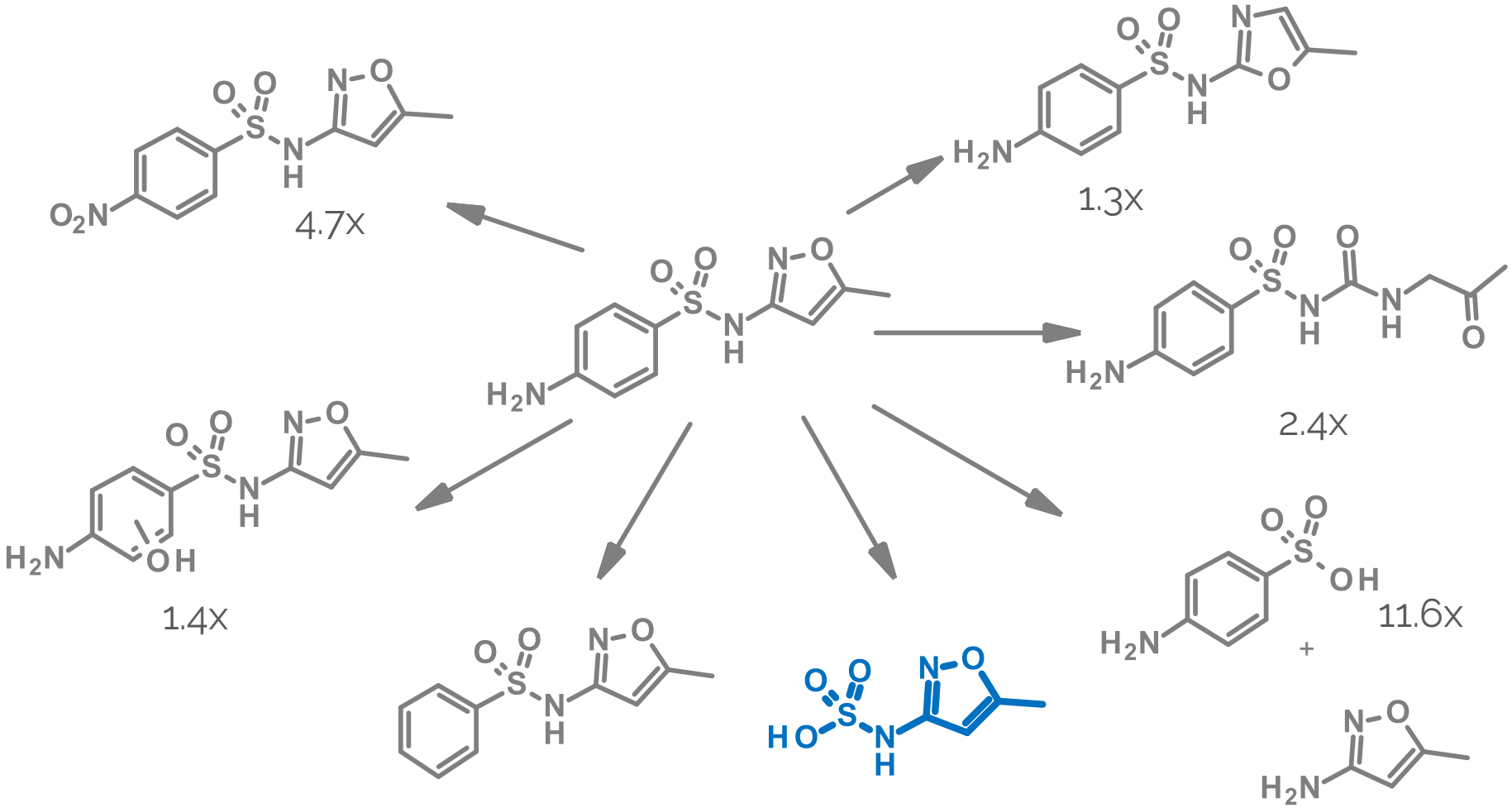


QUANTITATION STRATAGIES

PARENT COMPOUND

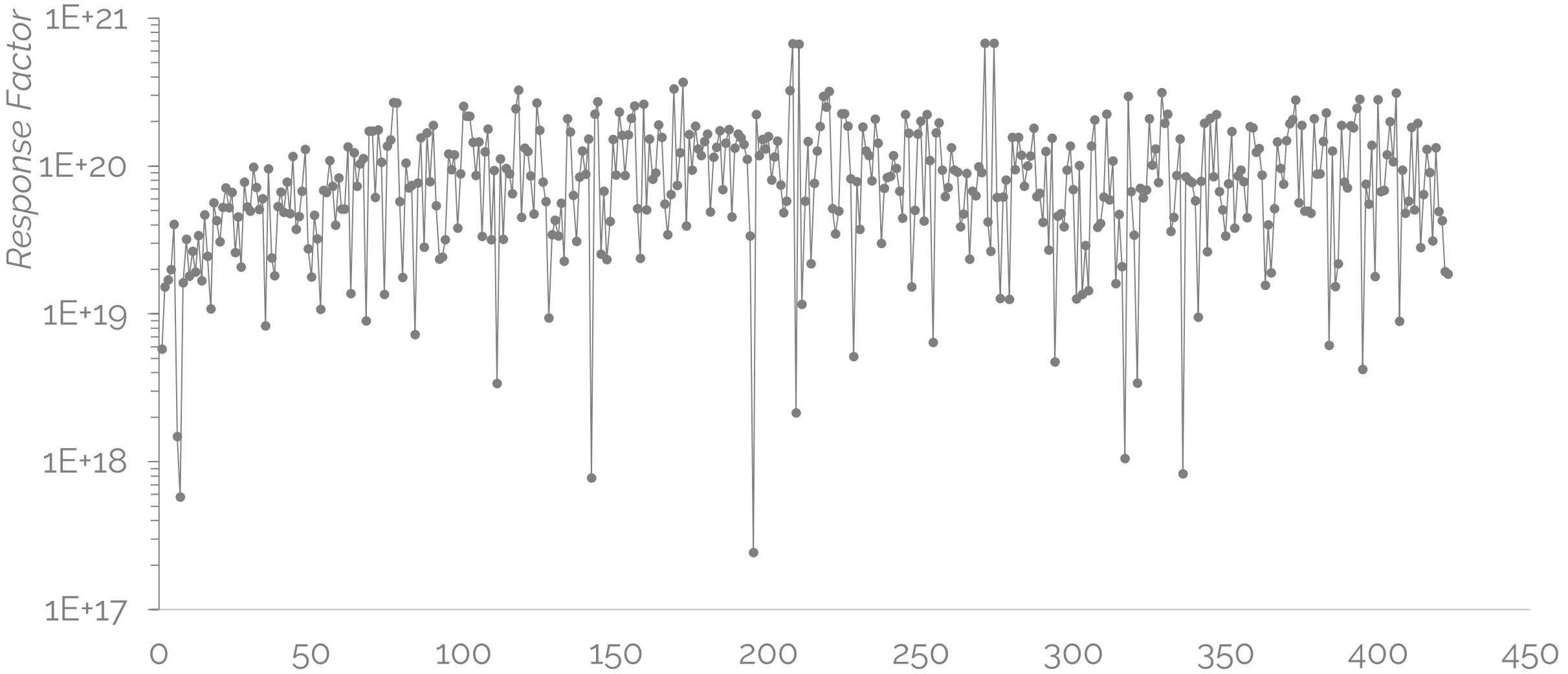
FOR CALIBRATION

up to 50 times lower ionization efficiencies relative to parent drug



COMPOUND ELUTING CLOSEST

TO THE COMPOUND OF INTEREST



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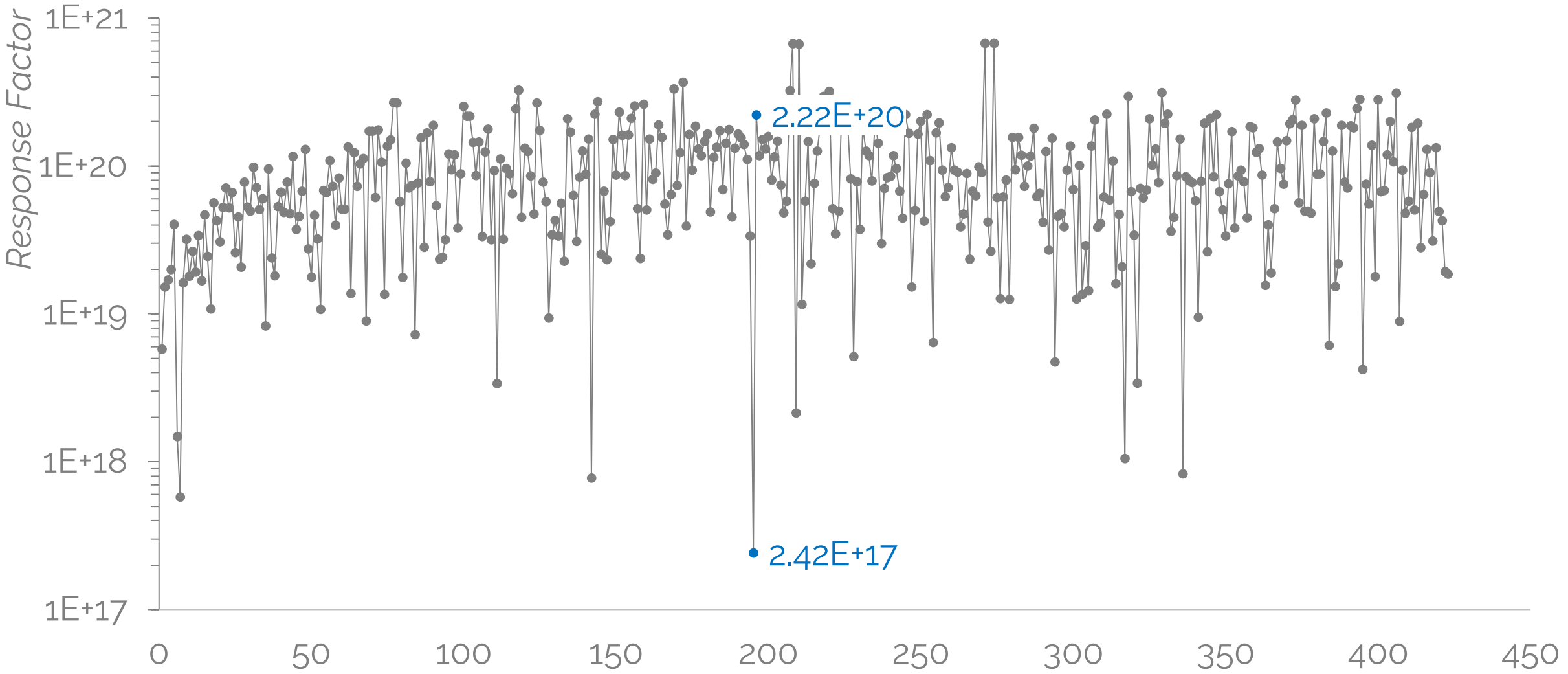


IONIZATION EFFICIENCY

FOR ESI SOURCE

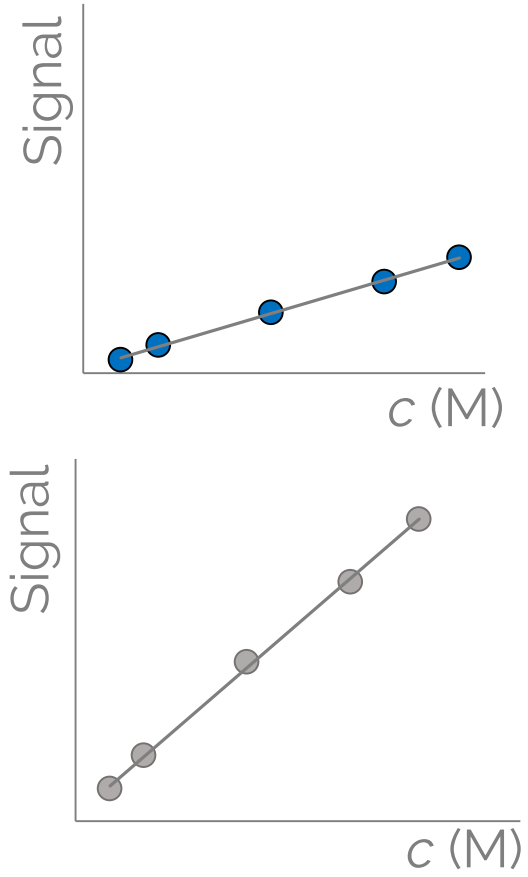
IONIZATION EFFICIENCY

RELATIVE MEASUREMENTS

flow injections

calibration graph

calculation



$$\frac{slope_1}{slope_2} \rightarrow IE$$

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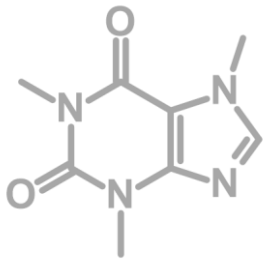
IONIZATION EFFICIENCY

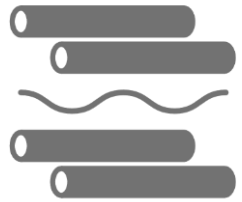
IS AFFECTED BY

structure



eluent

instrument



matrix

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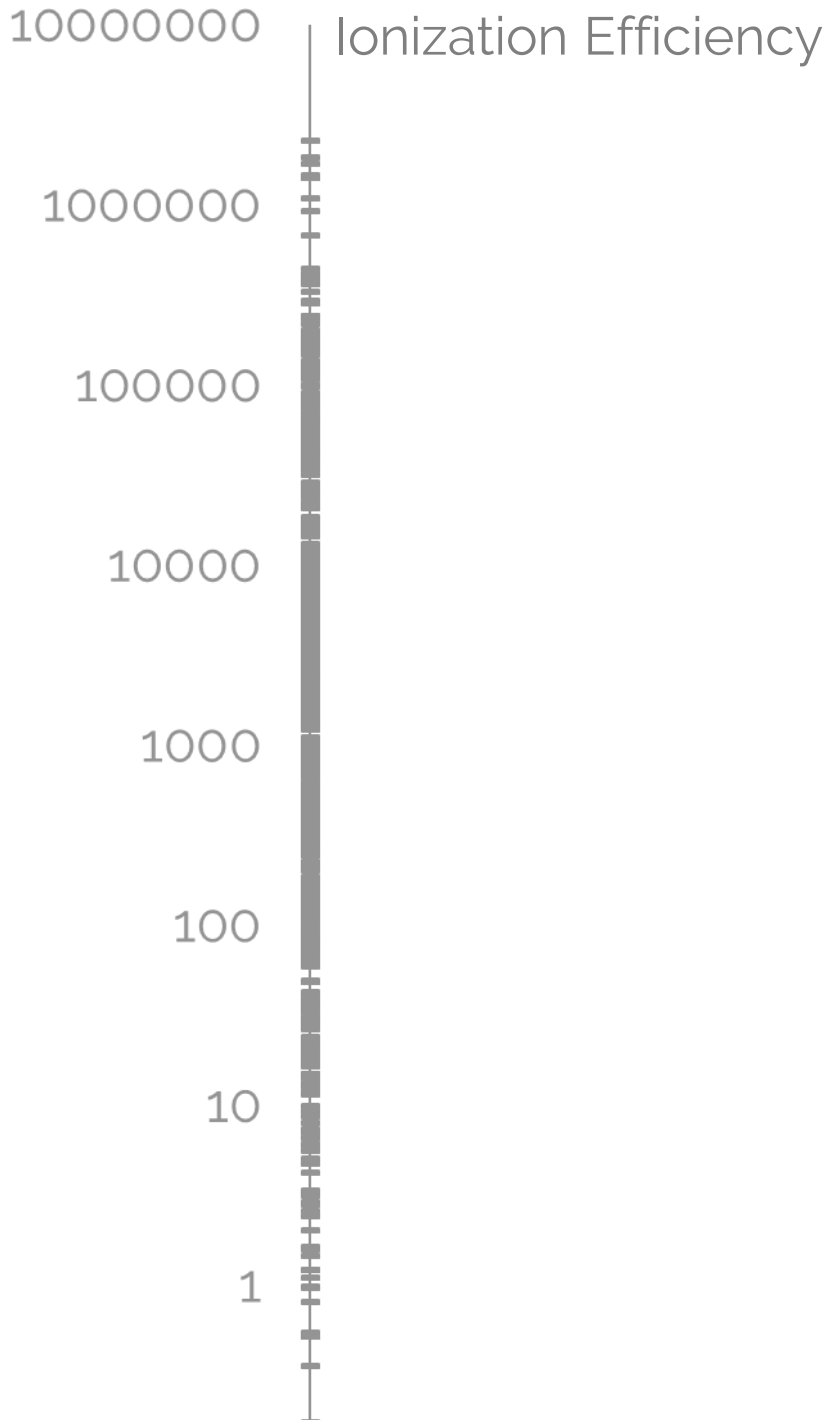
STRUCTURE

INTERPLAY BETWEEN PROPERTIES



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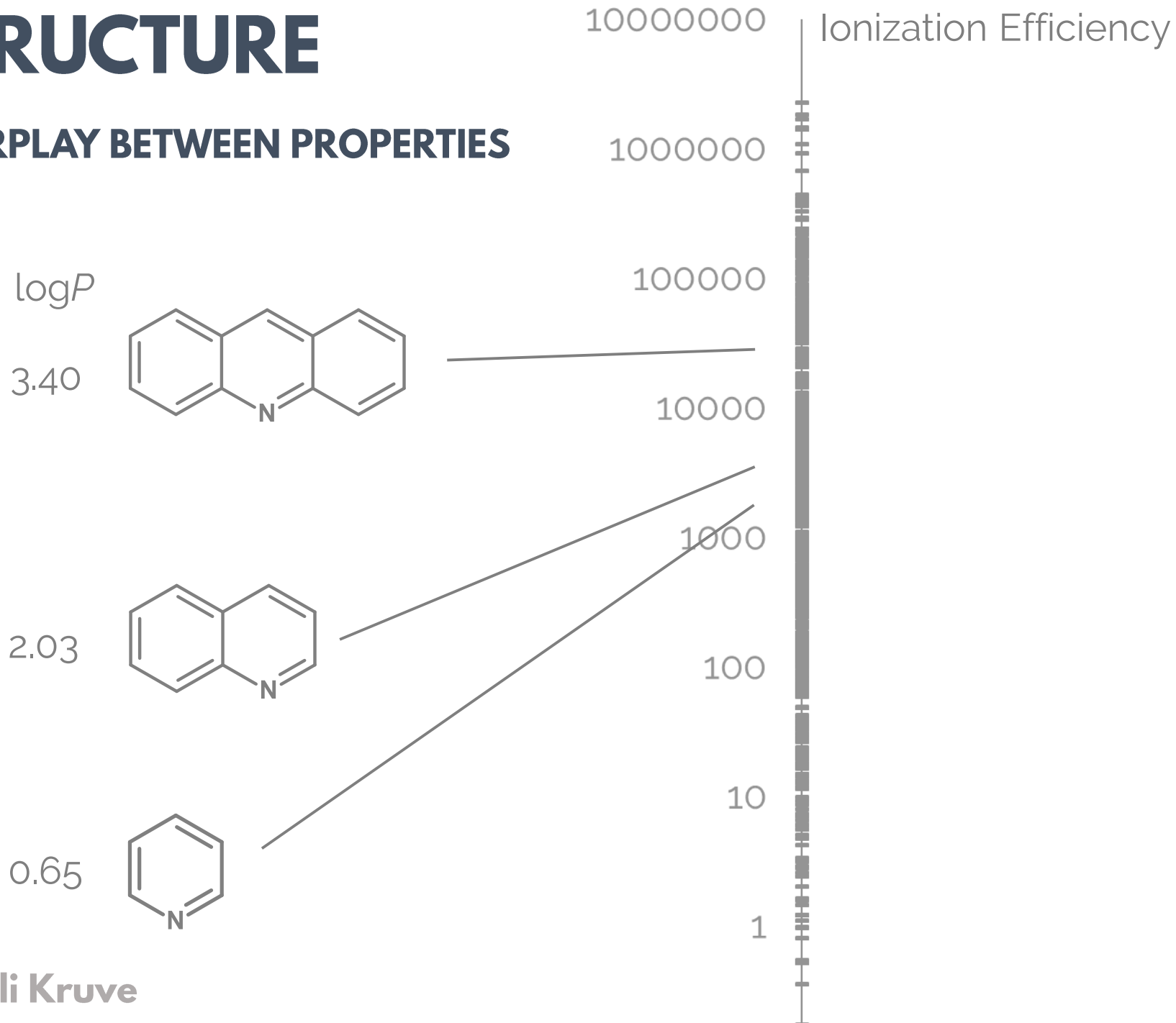
STRUCTURE

INTERPLAY BETWEEN PROPERTIES



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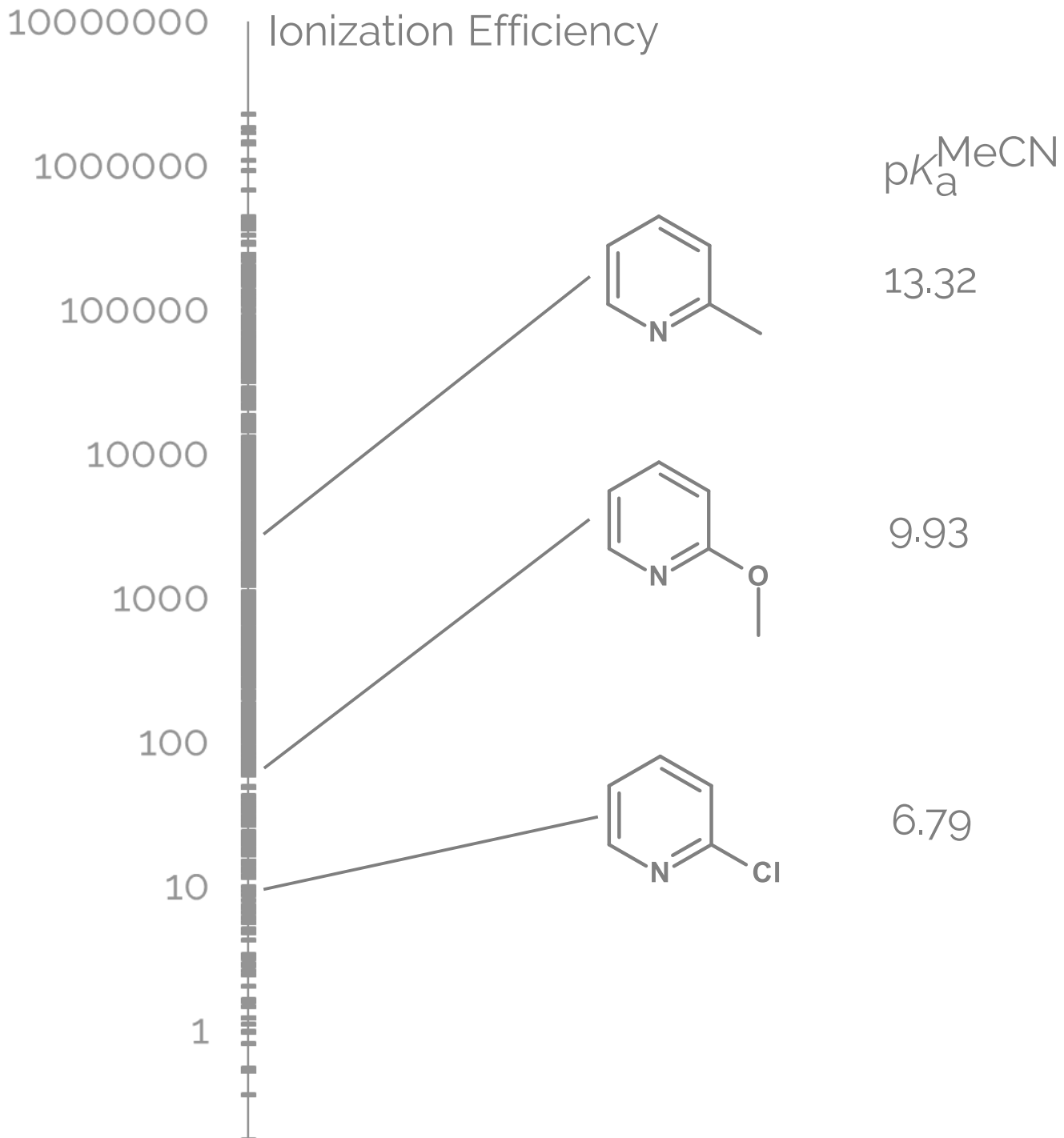
STRUCTURE

INTERPLAY BETWEEN PROPERTIES



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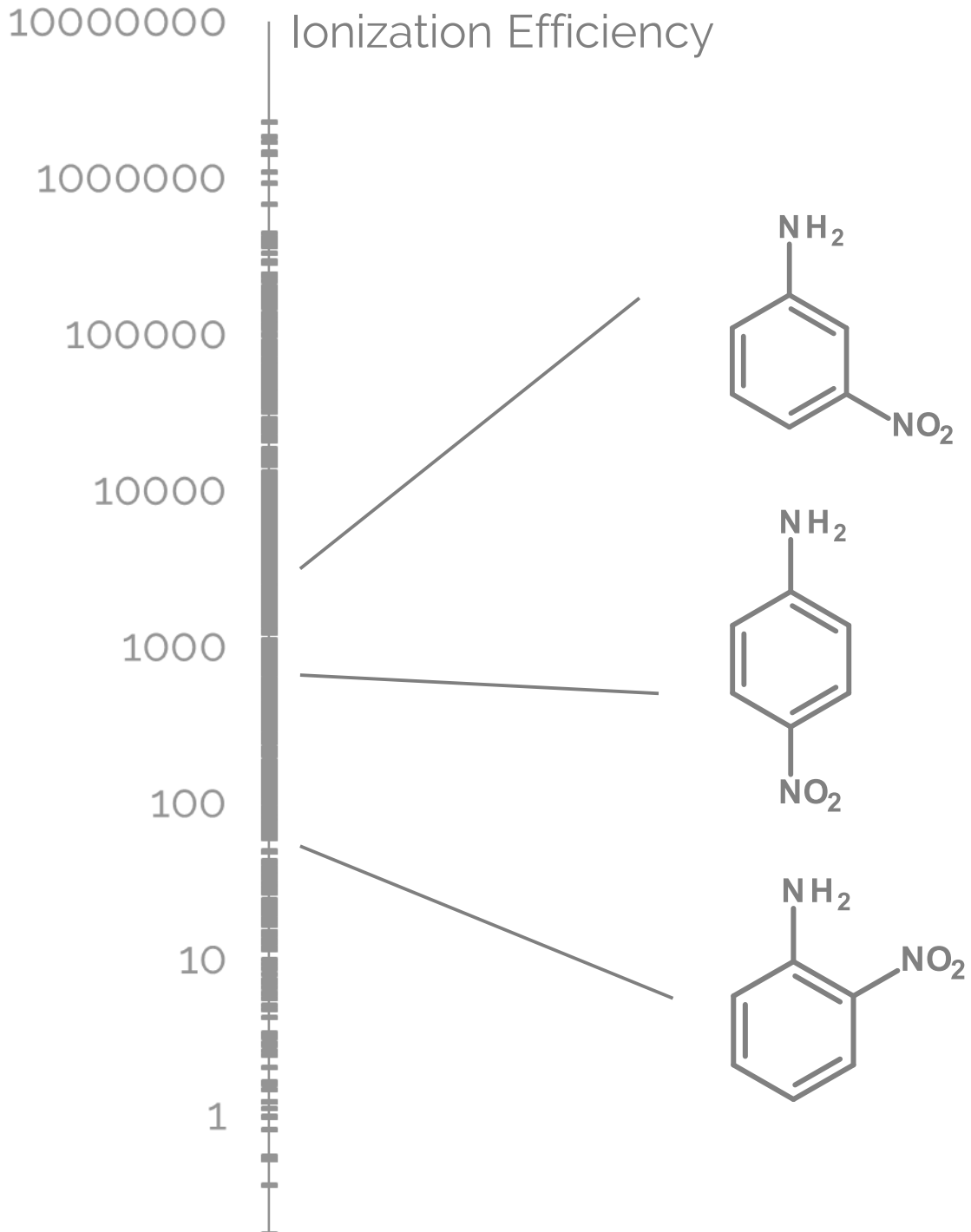
STRUCTURE

INTERPLAY BETWEEN PROPERTIES



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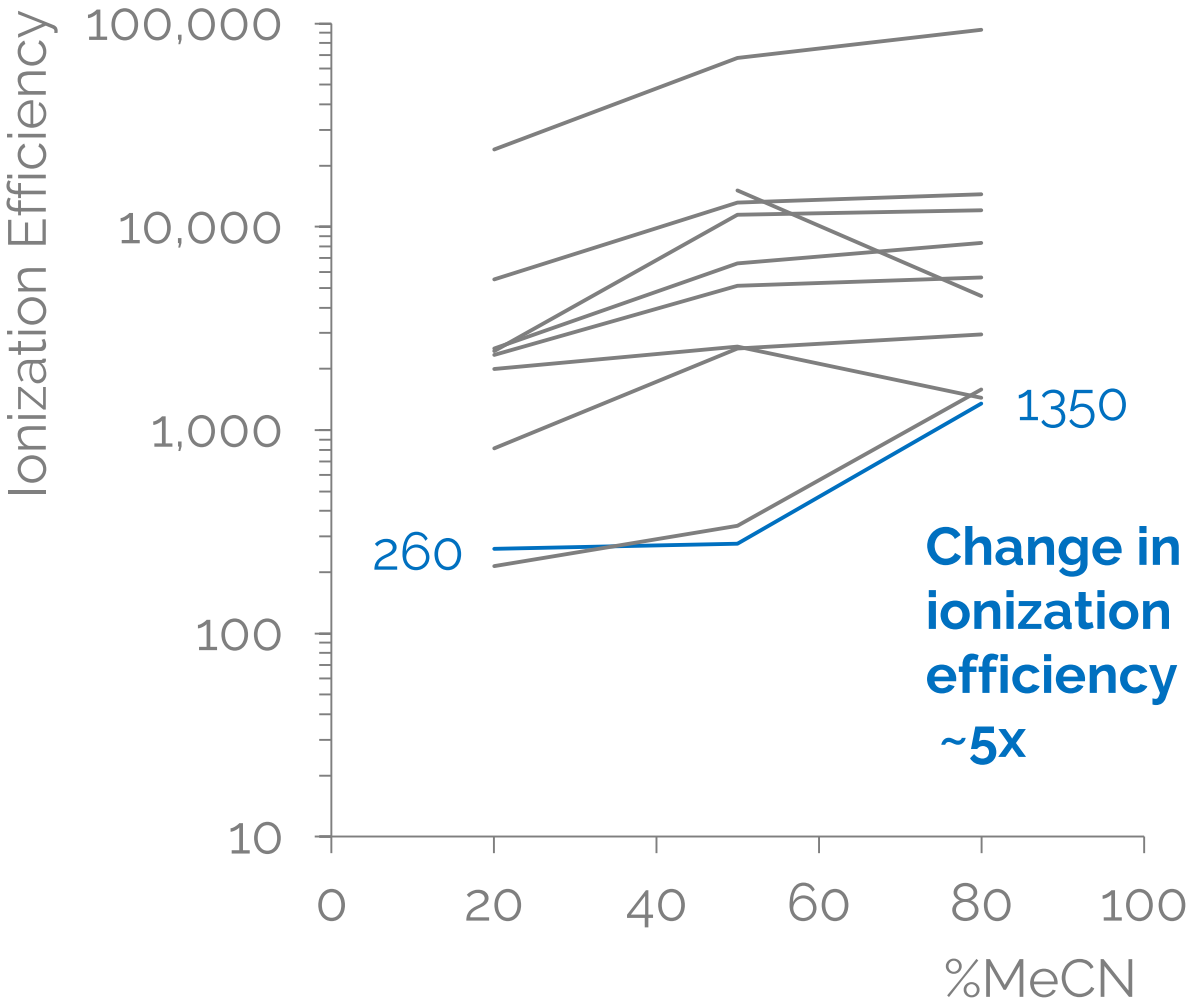
SOLVENT

ORGANIC SOLVENT CONTENT AND pH HAVE STRONG EFFECT ON IONIZATION EFFICIENCY



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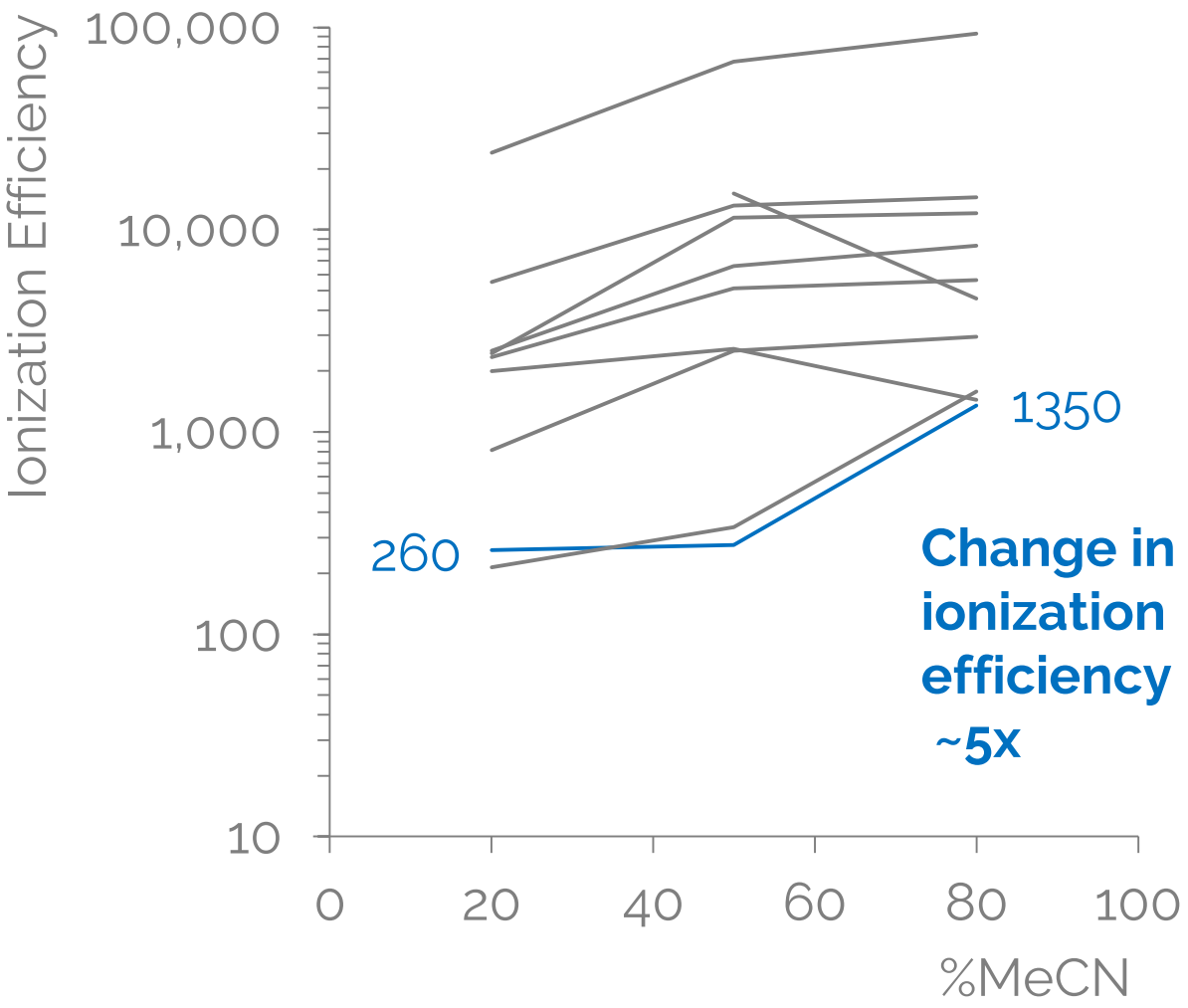
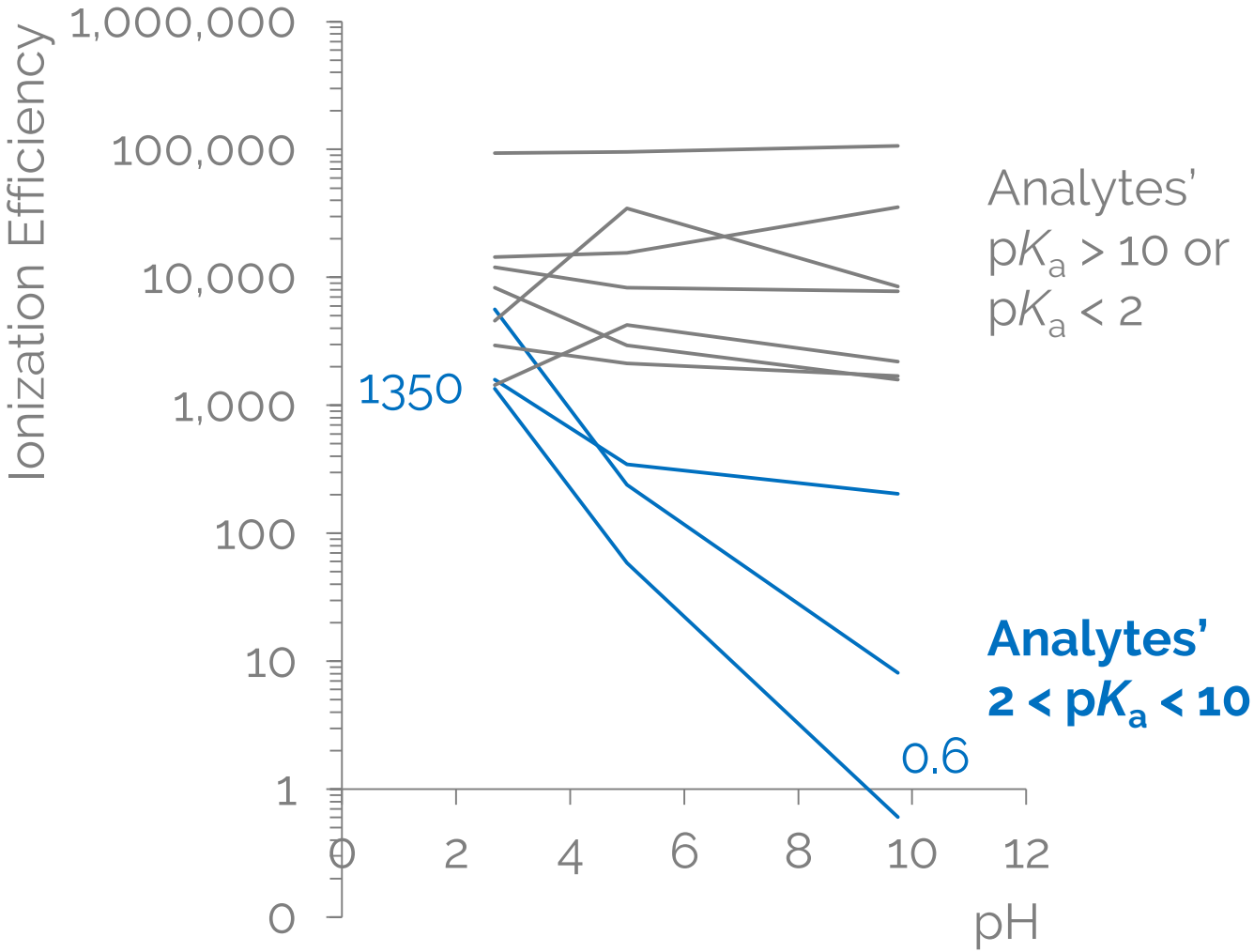
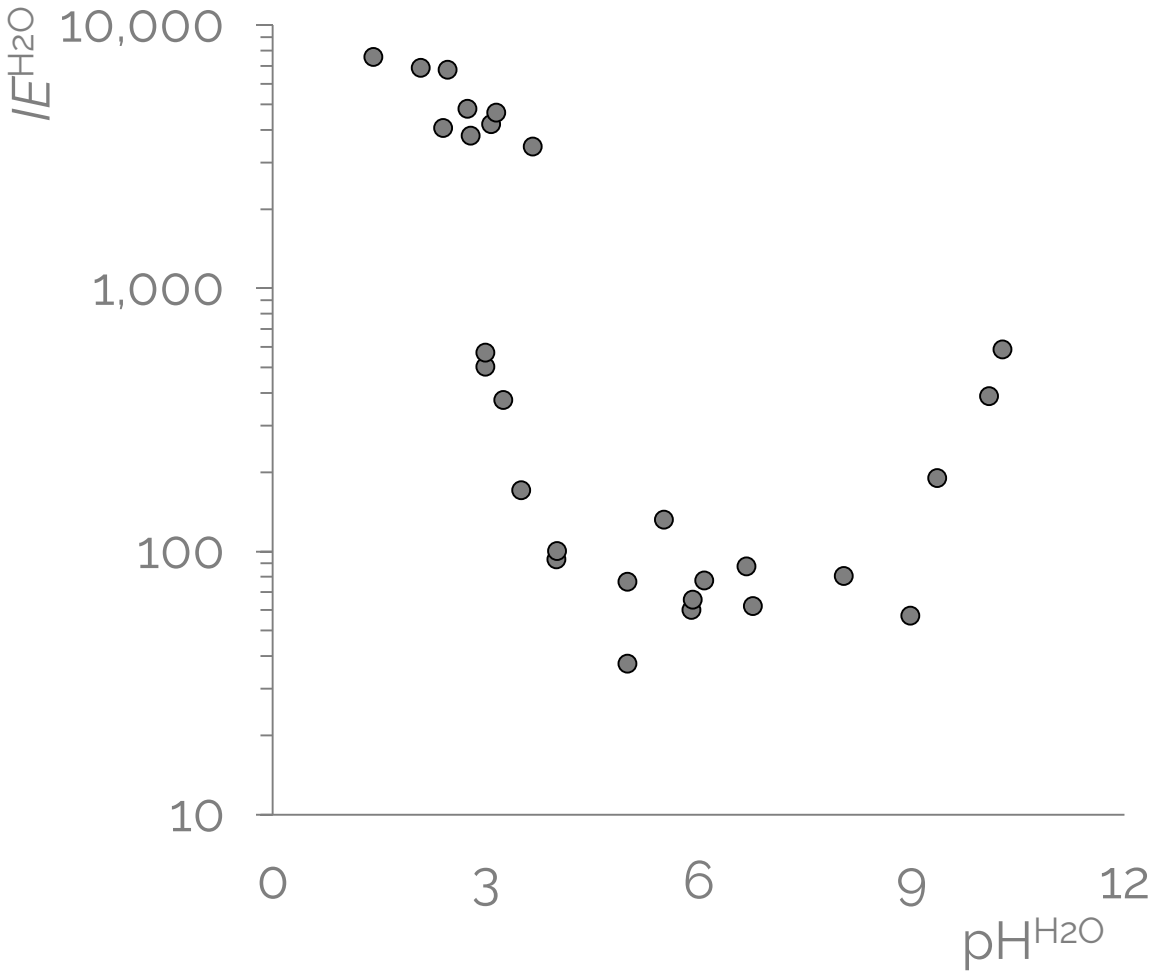
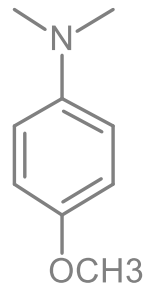




ADDITIVE EFFECT

WATER DROPLETS





$pK_a{}^{H2O} = 5.8$

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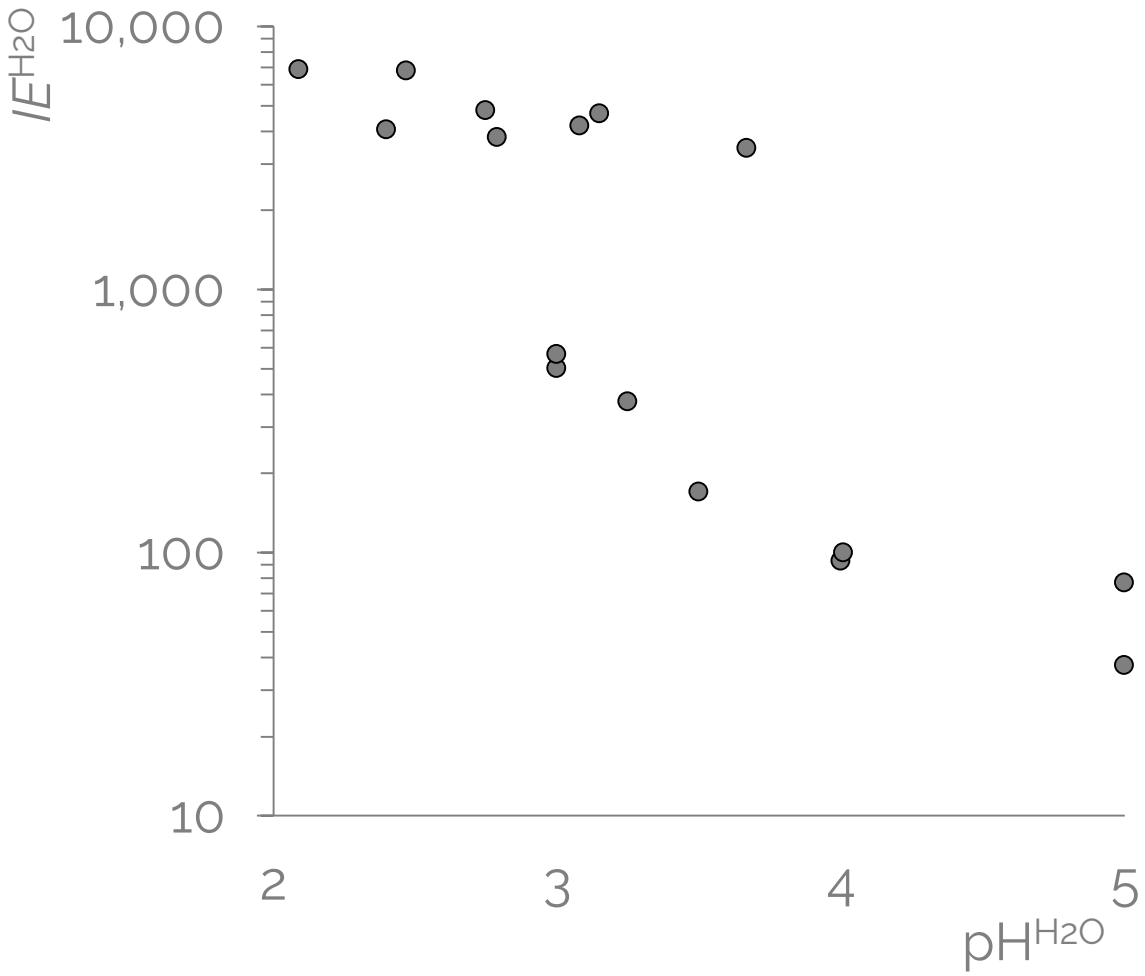
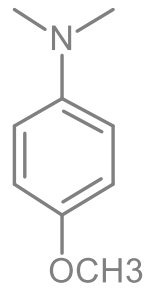
ADDITIVE EFFECT

WATER DROPLETS





$pK_a^{H2O} = 5.8$

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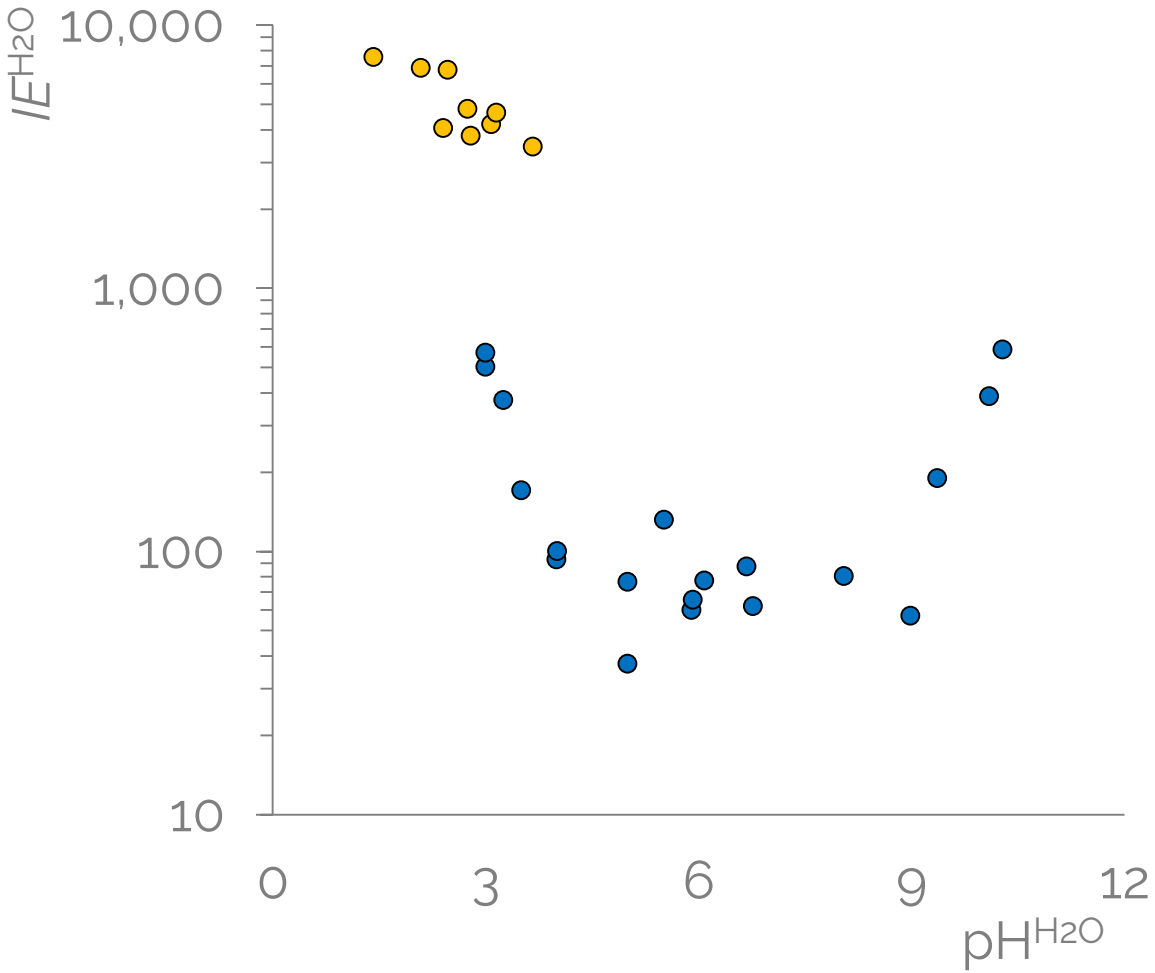
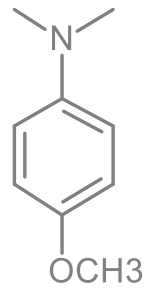
ADDITIVE EFFECT

WATER DROPLETS

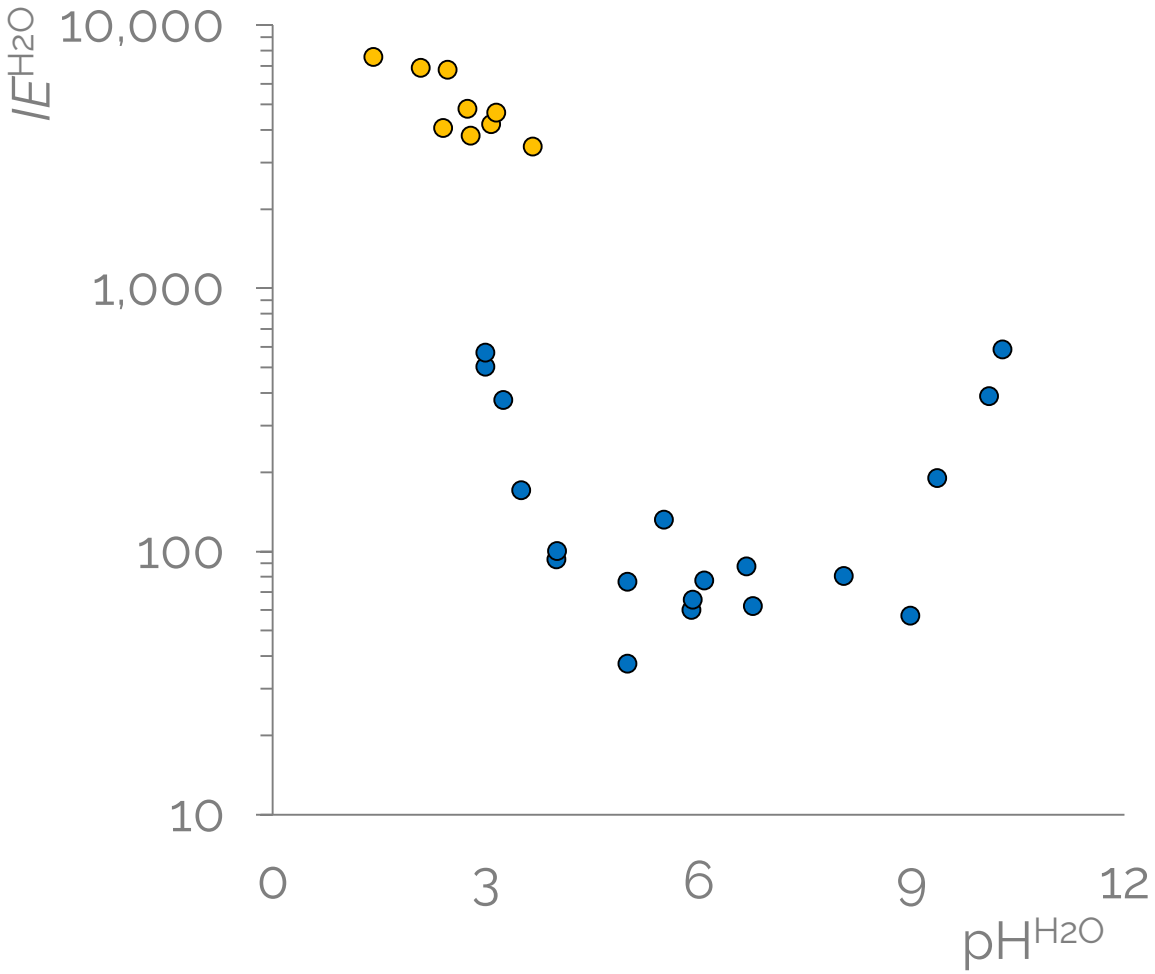


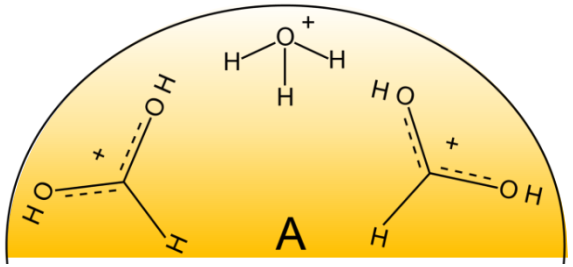


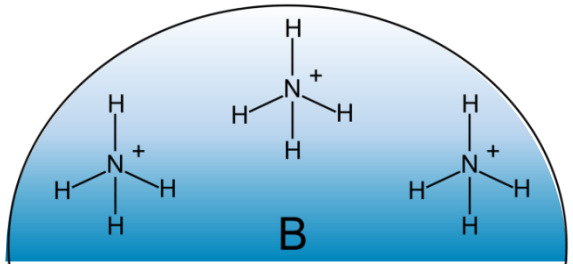
$pK_a^{H2O} = 5.8$

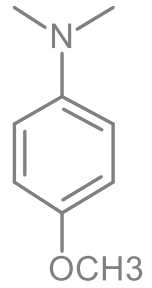
ADDITIVE EFFECT

WATER DROPLETS









$pK_a^{H2O} = 5.8$

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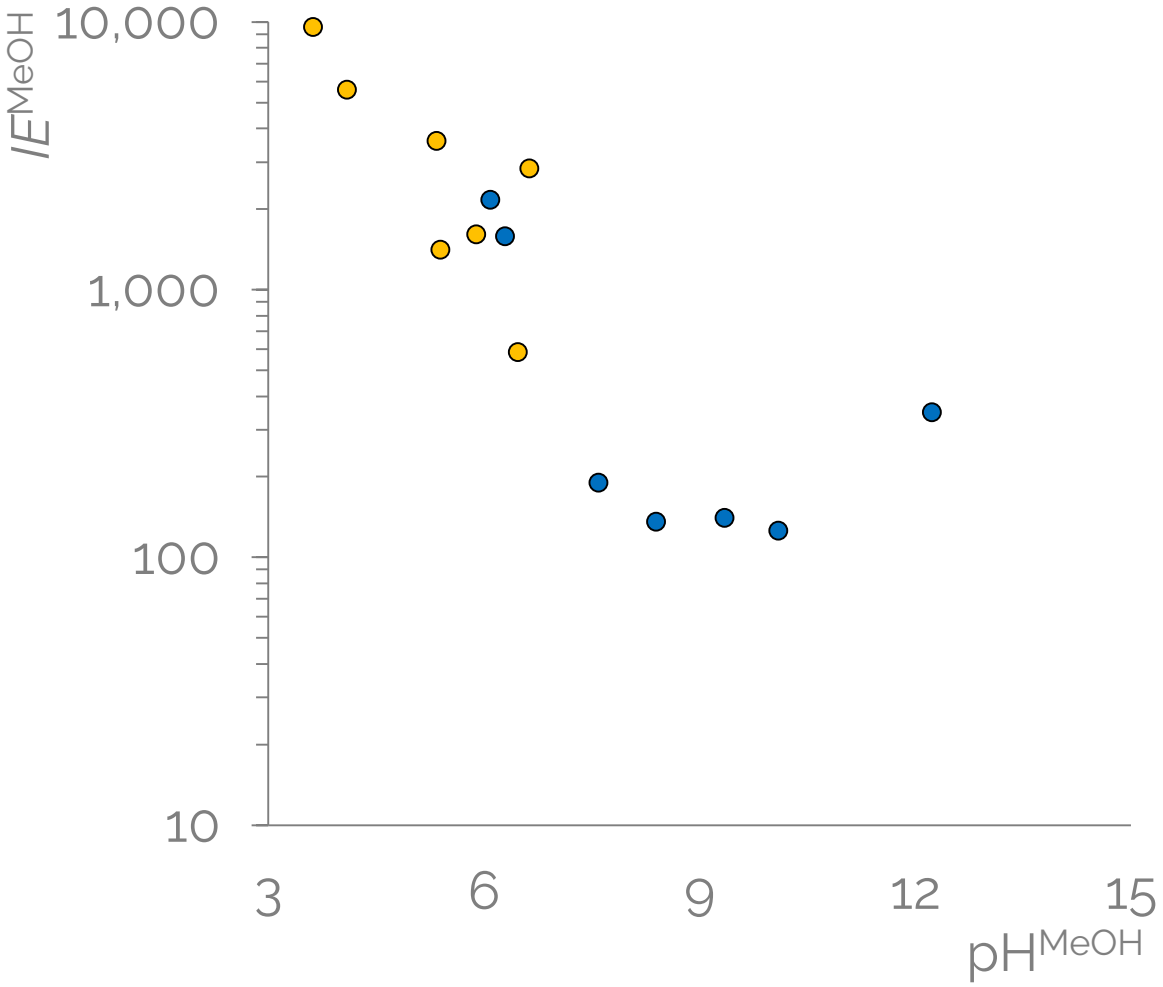
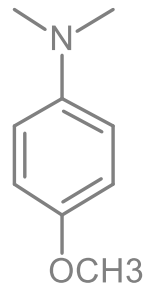
ADDITIVE EFFECT

METHANOL DROPLETS





$pK_a^{H2O} = 5.8$

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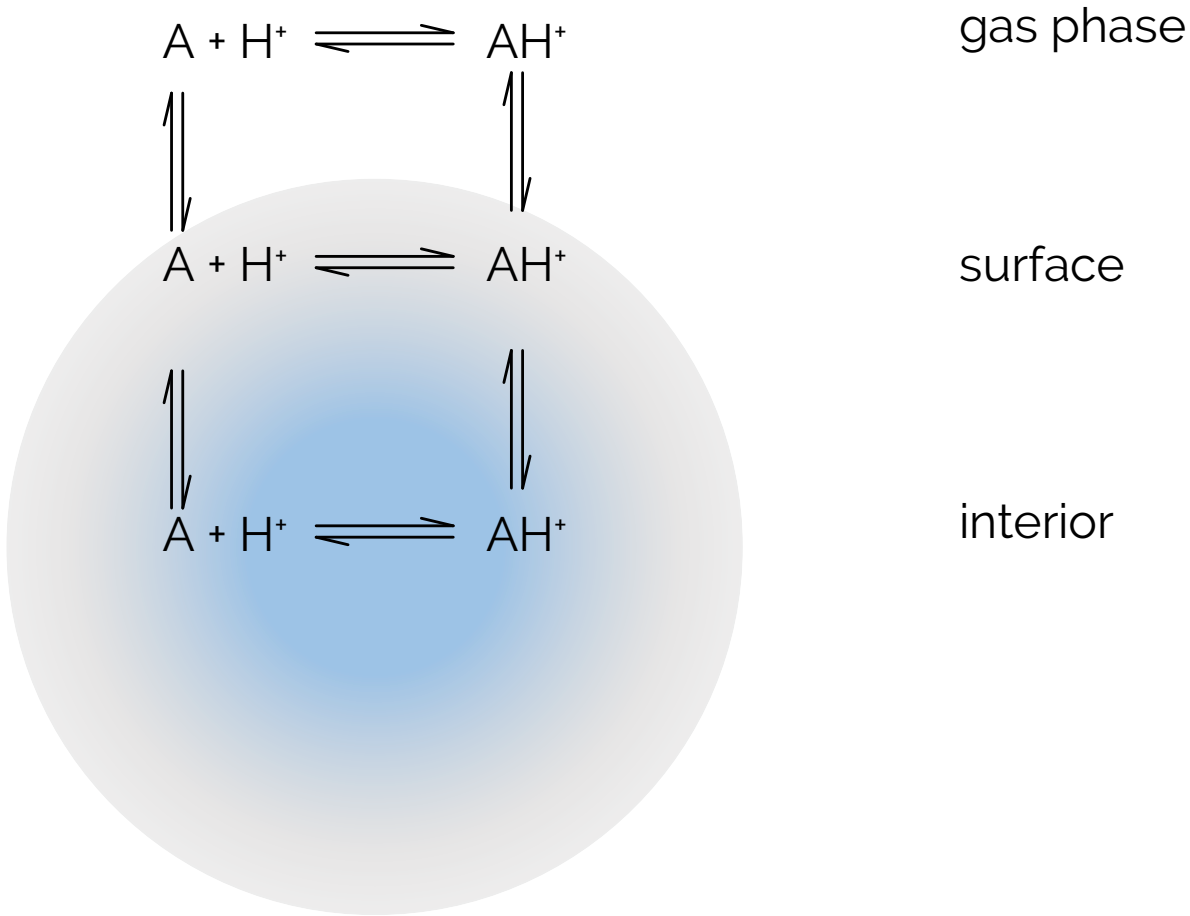
UNDERSTANDING

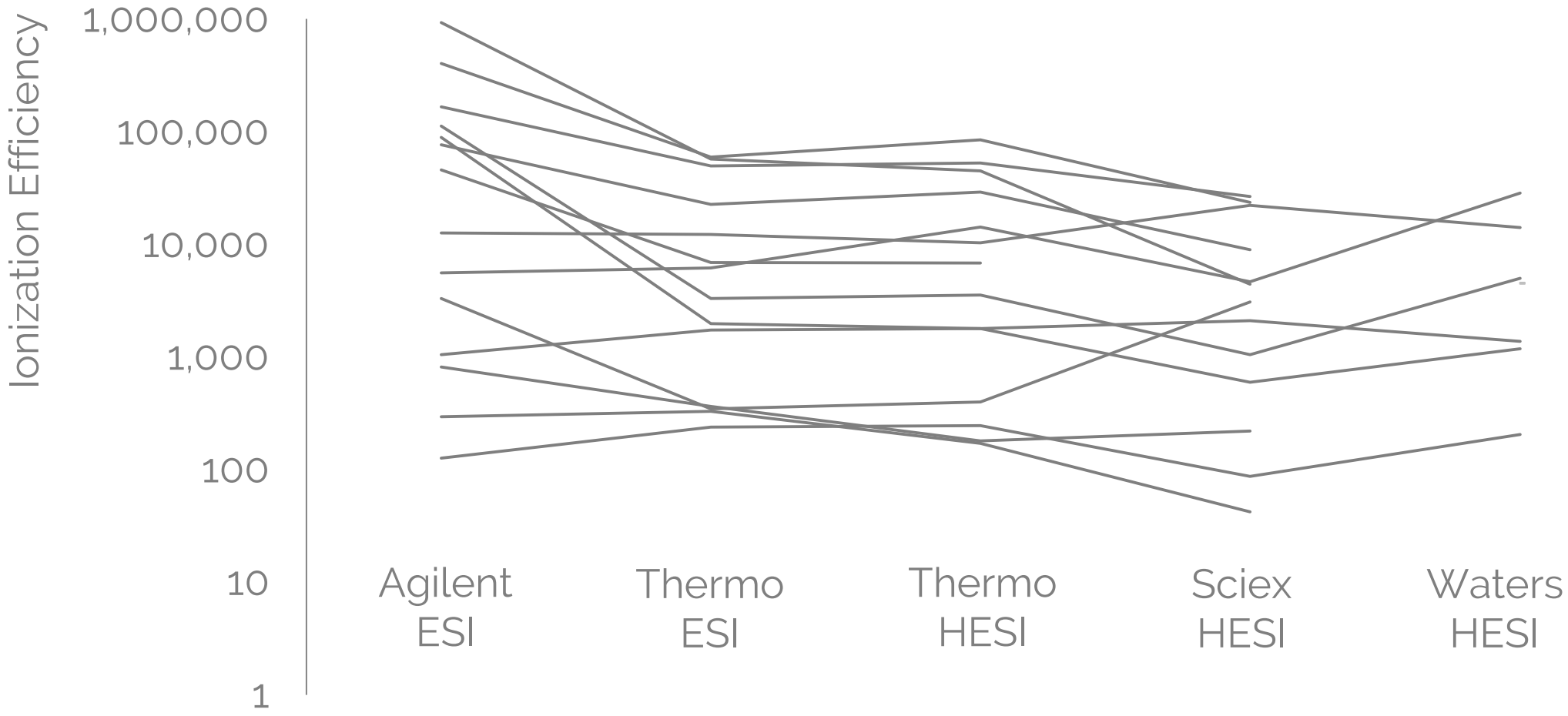
THE MECHANISM



INSTRUMENT

INFLUENCES IONIZATION EFFICIENCY

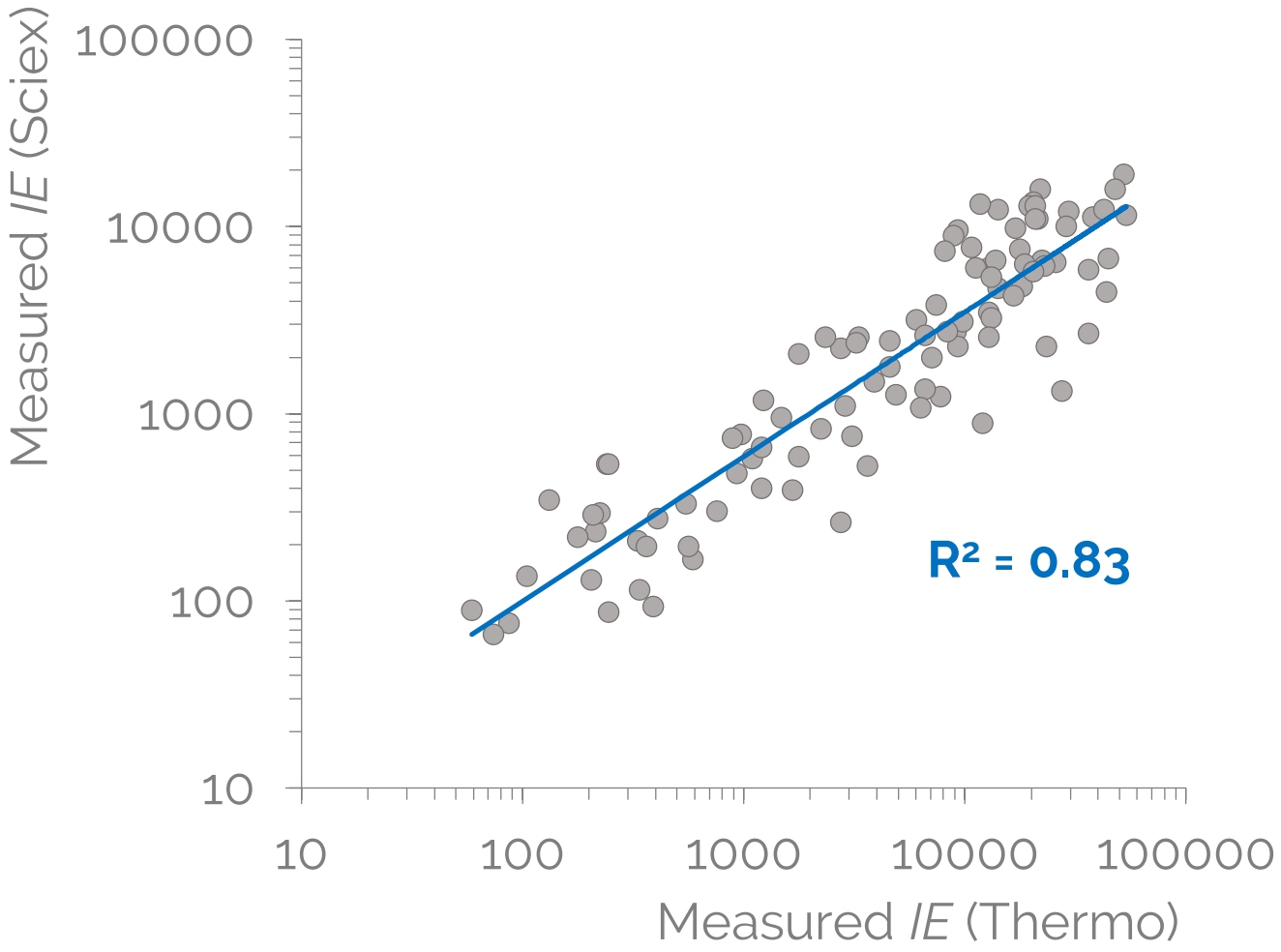
good correlation observed



INSTRUMENT

INFLUENCES IONIZATION EFFICIENCY

good correlation observed



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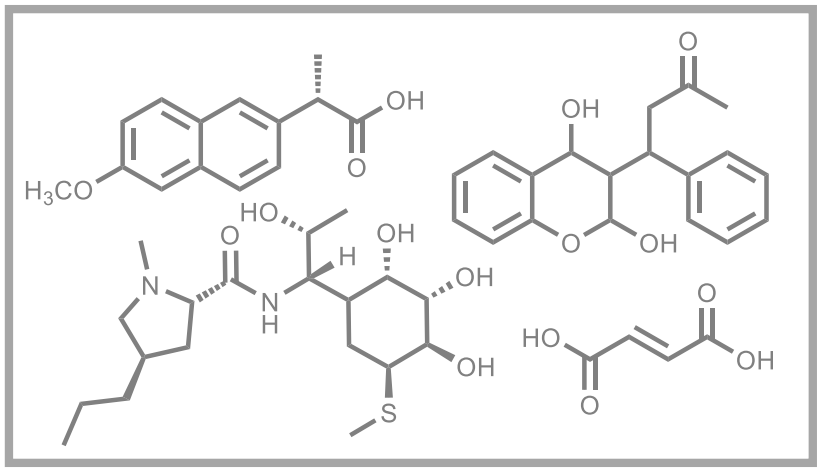
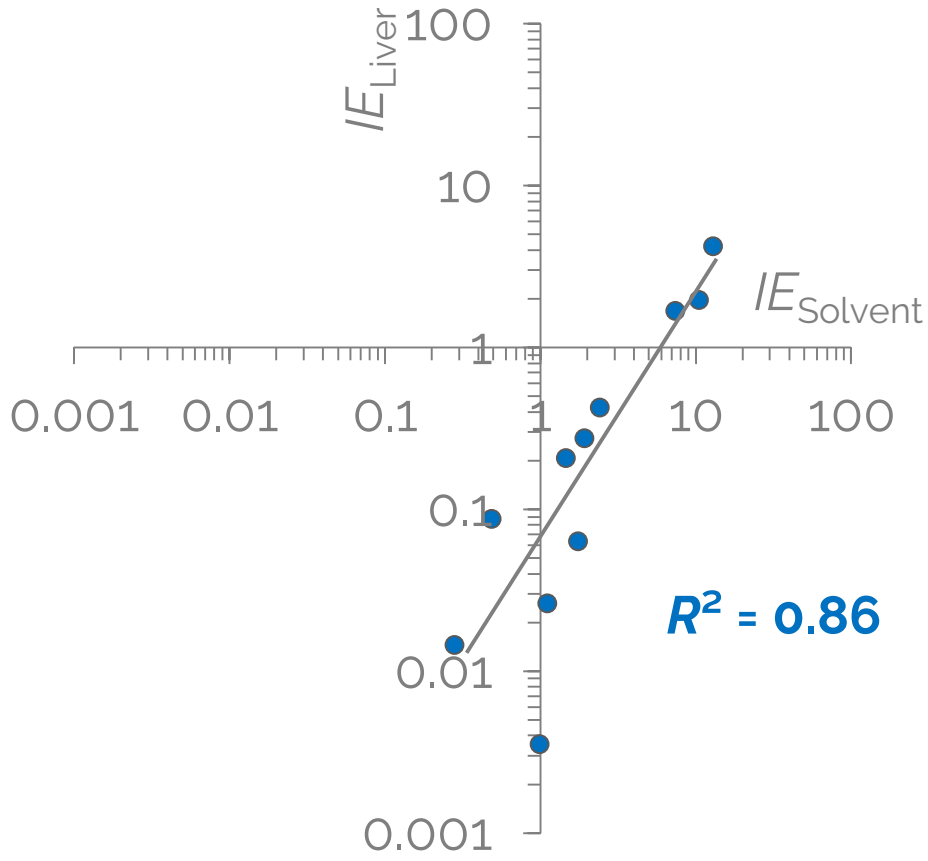
BIOLOGICAL MATRICES

SUPPRESS IONIZATION EFFICIENCY

good correlation with ionization efficiencies in solvent





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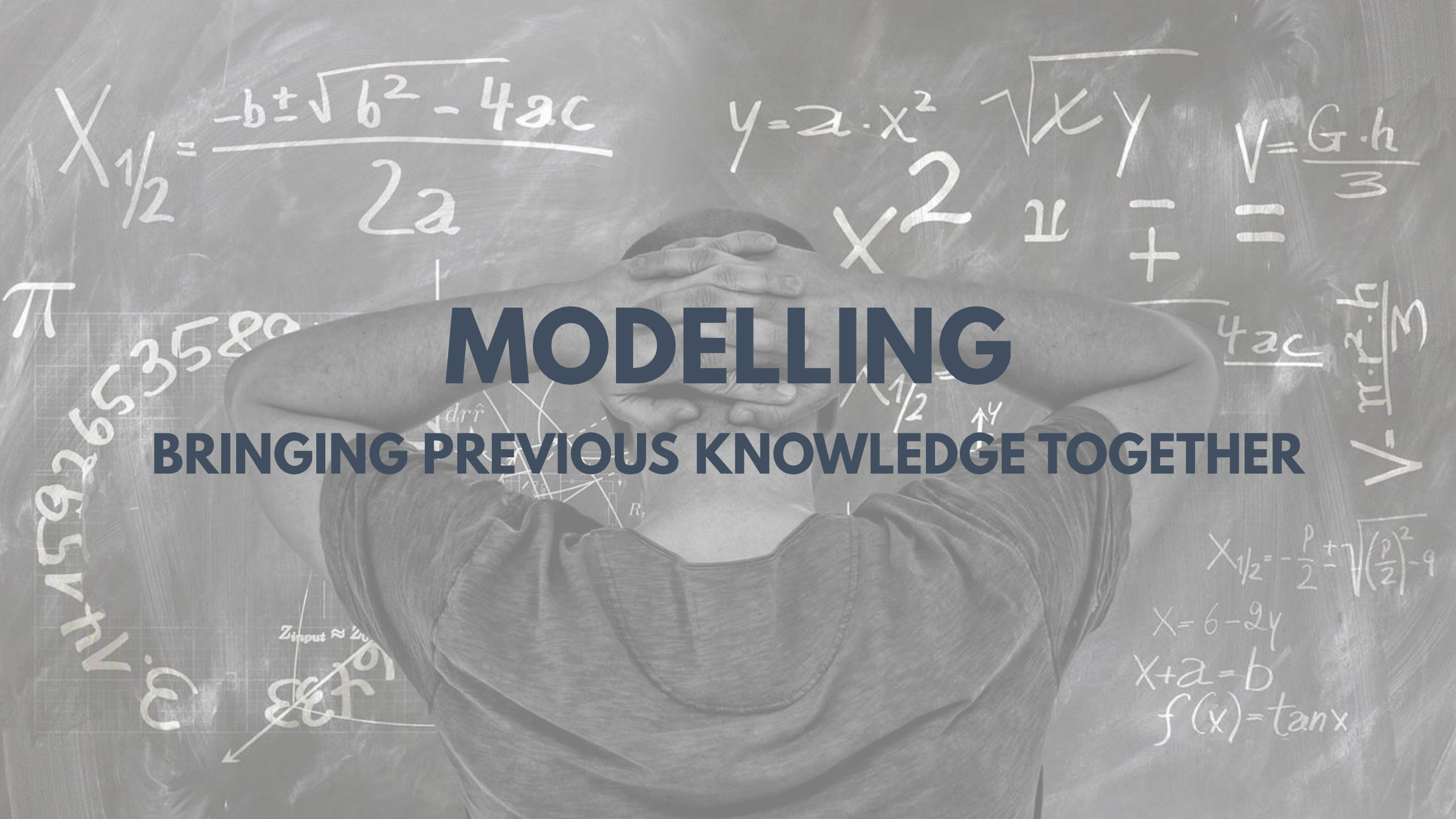
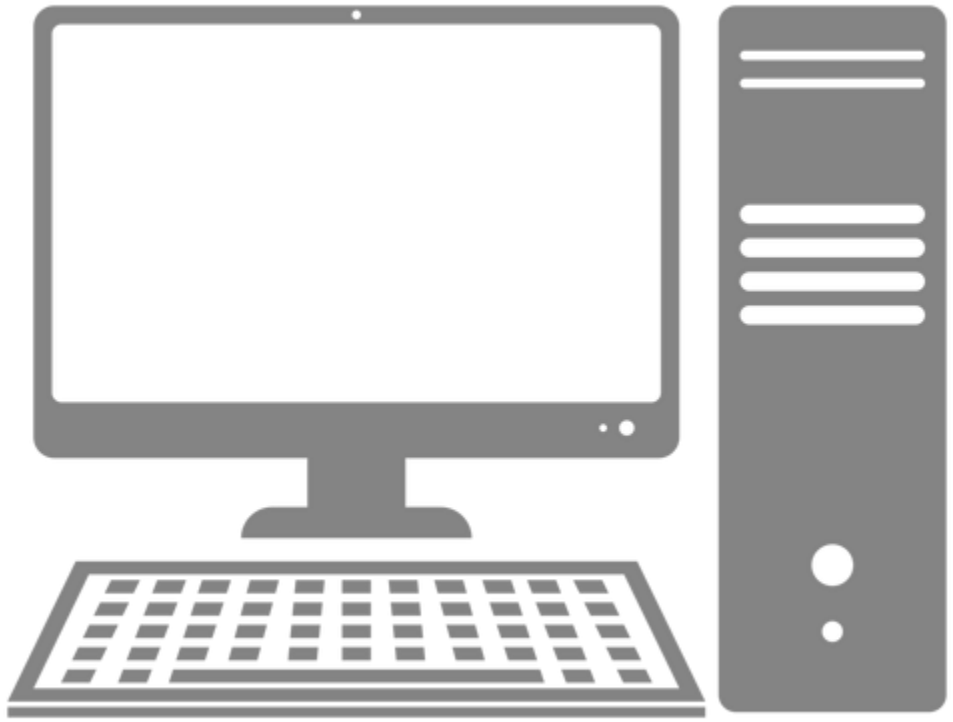


MODELLING

BRINGING PREVIOUS KNOWLEDGE TOGETHER

MACHINE LEARNING



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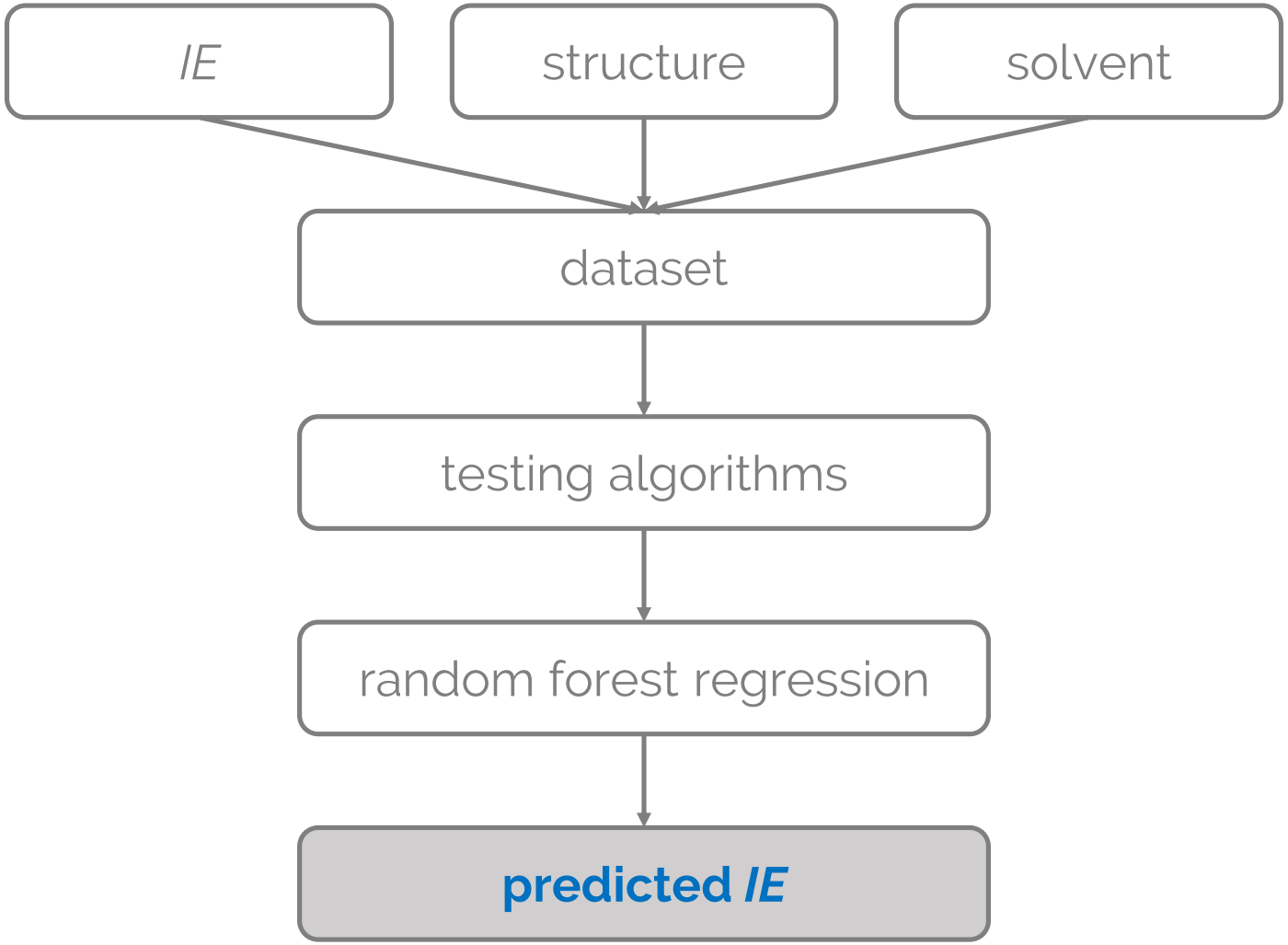
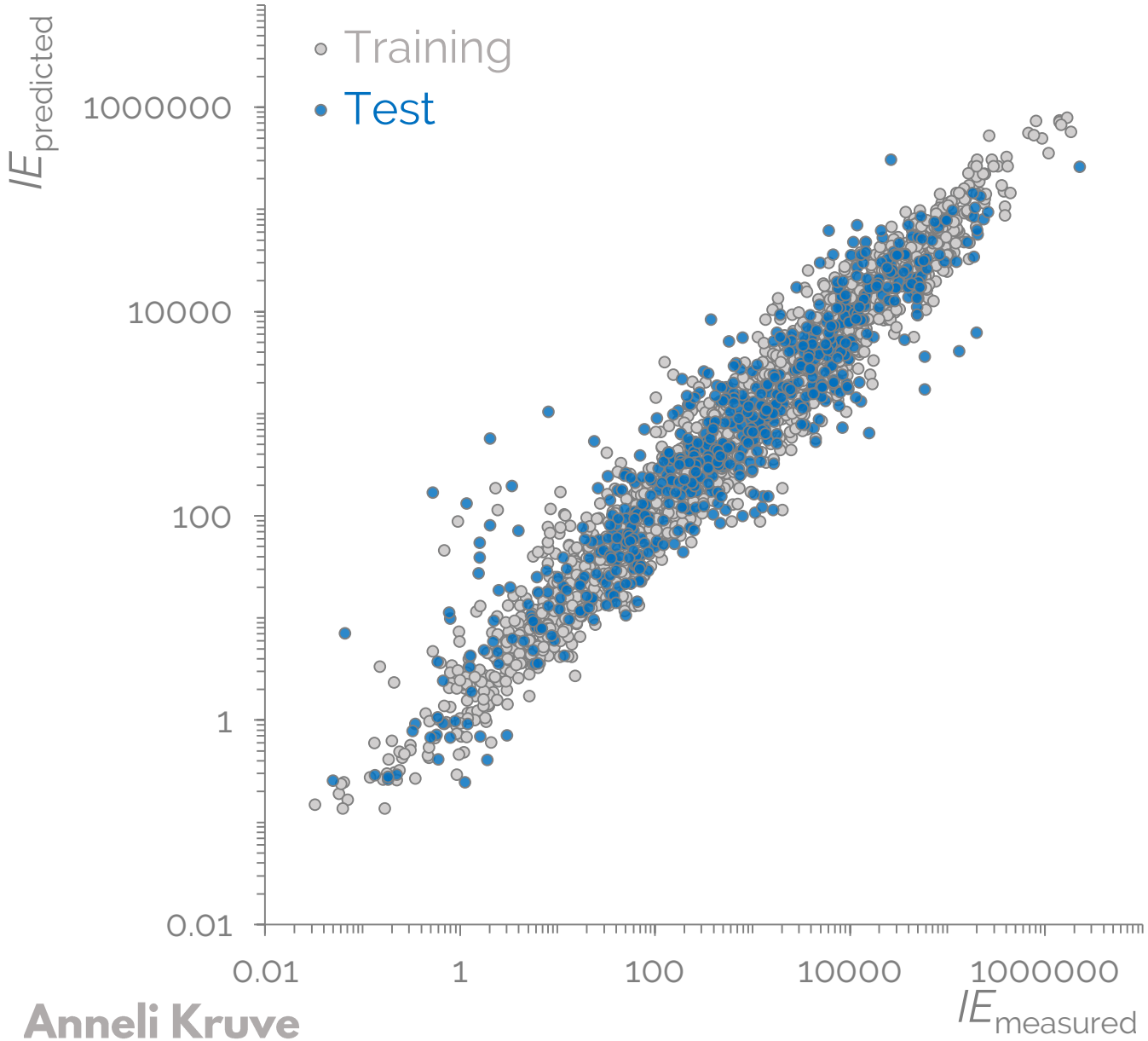
MODELLING OF IONIZATION



PREDICTIONS IN ESI+



3139 data points

353 small molecules

106 eluent compositions

MeCN/MeOH

0 – 100%

pH = 2.0 – 10.7

Prediction error

Training set **1.9x**

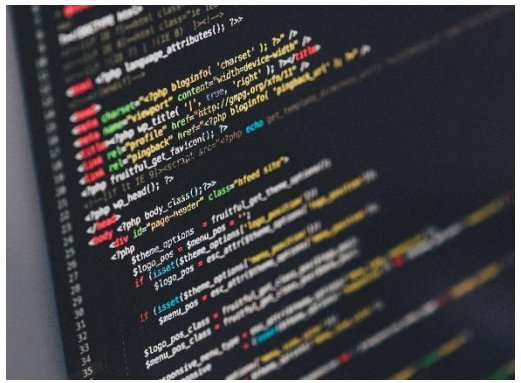
Test set **3.0x**

HOW TO USE IT?

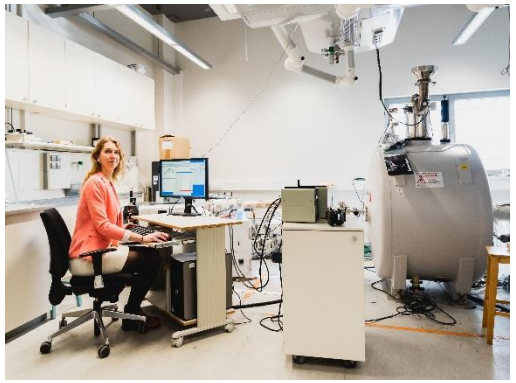
FOR QUANTIFICATION

in any given lab on any given instrument

predict



transfer



calculate

CONCENTRATIONS

PESTICIDES IN CEREALS

SAMPLES

PESTICIDES AND MYCOTOXINES

in oat, barley, rye, wheat, rice, maize

35 compounds + compounds with known concentration

concentrations 3.6 nM – 0.35 mM

measured on a QQQ

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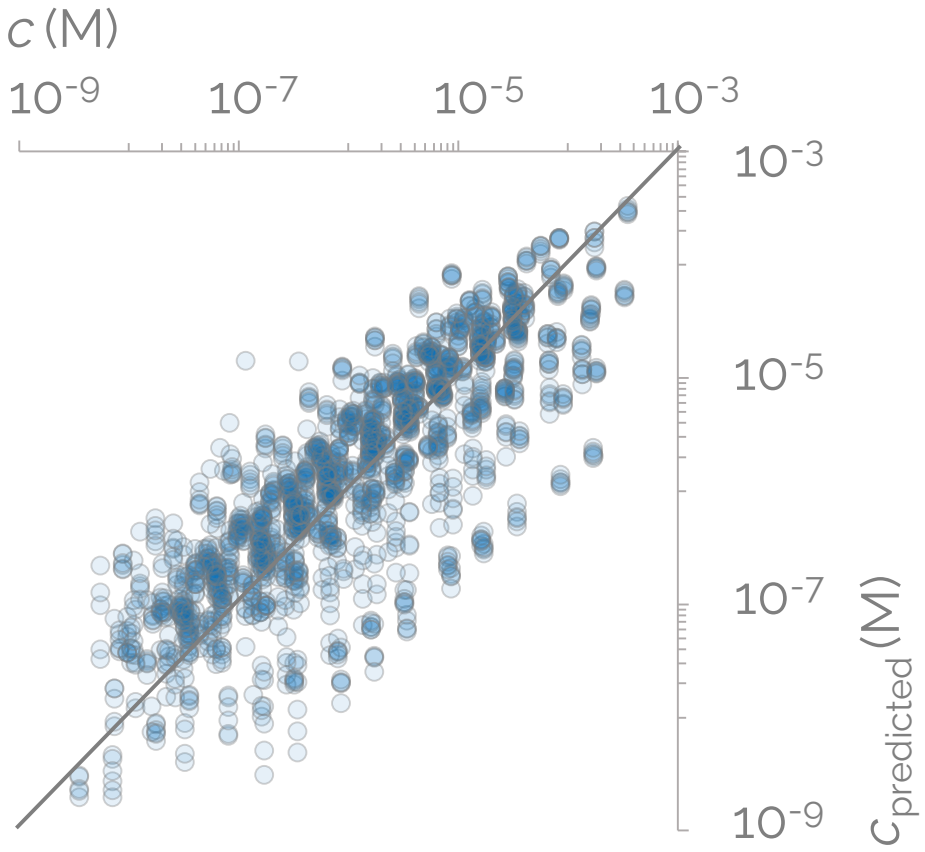
QUANTIFICATION

VALIDATION

for 35 pesticides in cereal samples



2233 data points

Mean prediction error 5.3x

89% of points with accuray better than 10 fold

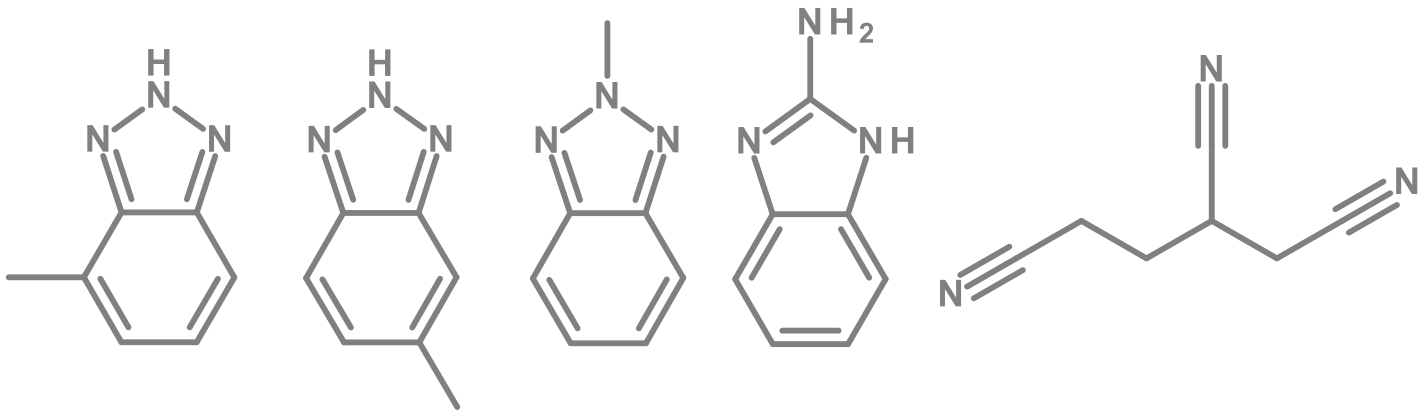
CONCENTRATIONS

OF ENVIRONMENTAL CONTAMINANTS

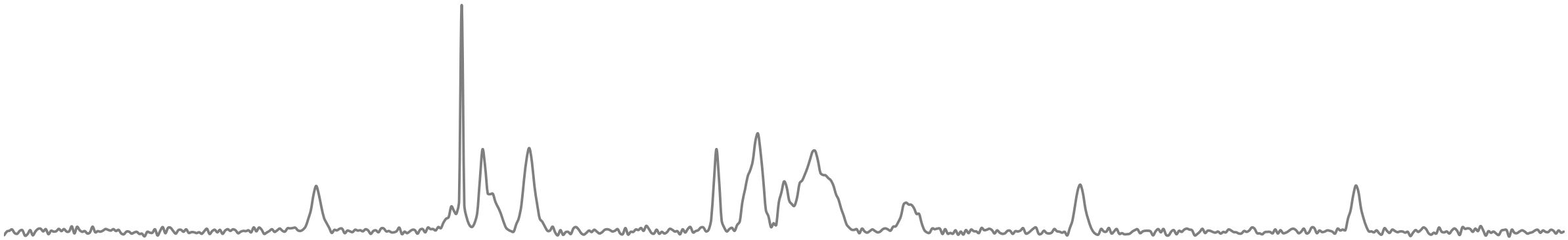
PRIORITIZATION

BASED ON TOXICITY

ToxCast toxicity tests

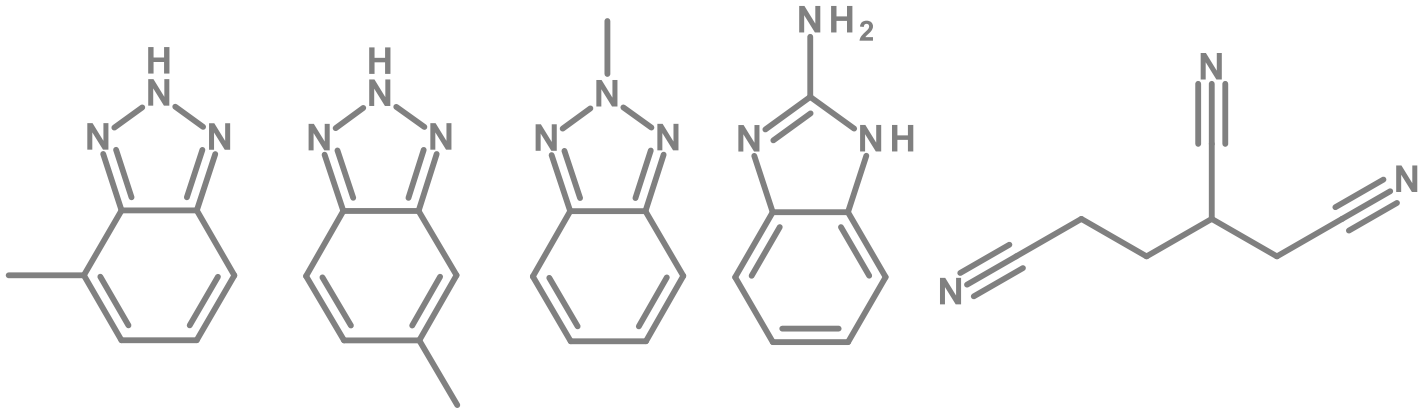


AC_{50} (µM)	NA	0.27	NA	12.82	NA

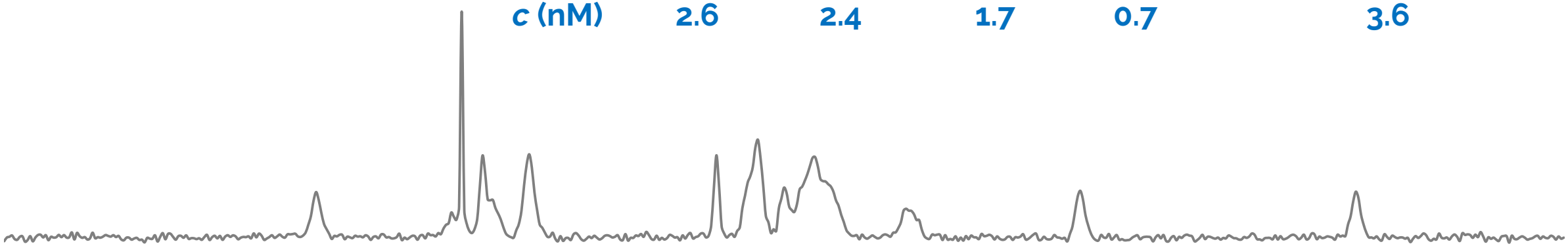


QUANTIFICATION

PREDICTED CONCENTRATION



AC_{50} (µM)	NA	0.27	NA	12.82	NA
***c* (nM)**	**2.6**	**2.4**	**1.7**	**0.7**	**3.6**



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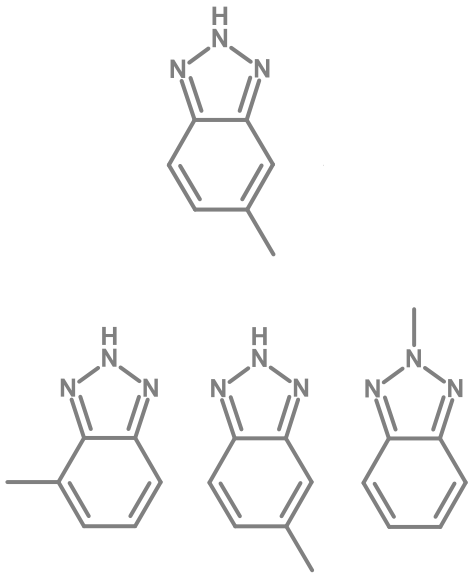
IDENTIFICATION LEVELS

Example	Identification confidence	Minimum data requirements
 $C_7H_7N_3$	Level 1: Confirmed structure by reference standard	MS, MS^2, RT, Reference Std.
	Level 2: Probable structure a) by library spectrum match b) by diagnostic evidence	MS, MS^2, Library MS^2 MS, MS^2, Exp. data
	Level 3: Tentative candidate(s) structure, substituent, class	MS, MS^2, Exp. data
	Level 4: Unequivocal molecular formula	MS isotope/adduct
133.0640	Level 5: Exact mass of interest	MS

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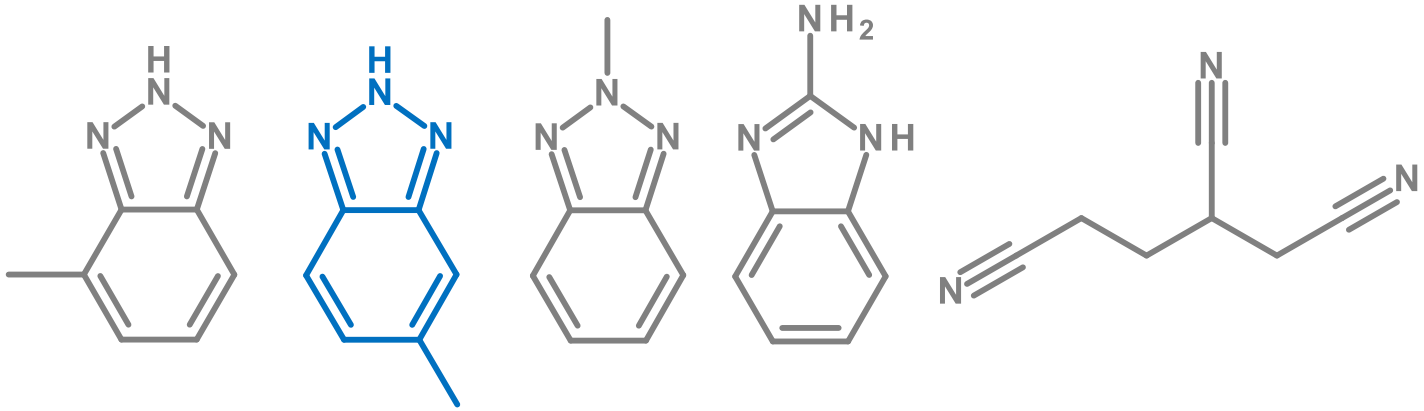
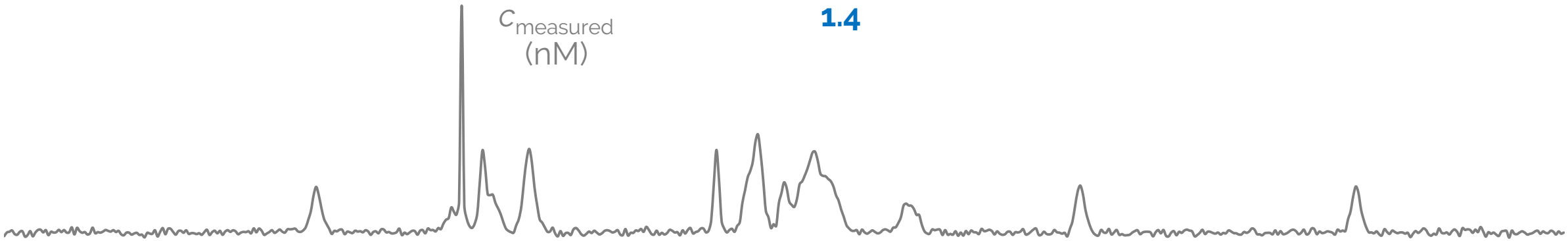
TARGETED METHODS

FOR CONFIRMATION AND QUANTIFICATION

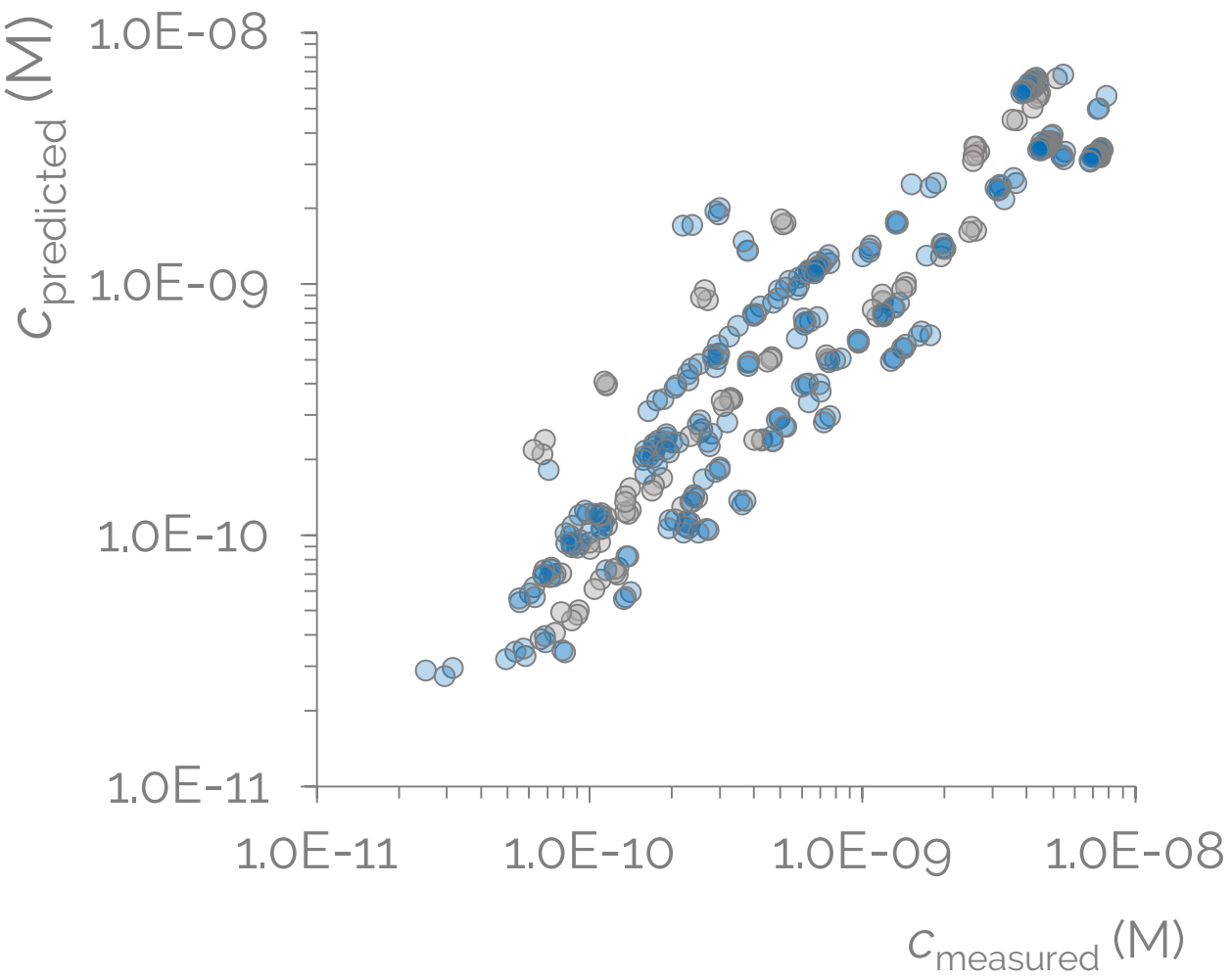


AC_{50} (µM)	NA	0.27	NA	12.82	NA
$c_{predicted}$ (nM)	2.6	**2.4**	1.7	0.7	3.6
$c_{measured}$ (nM)		**1.4**			



QUNATIFICATION OF CONTAMINANTS

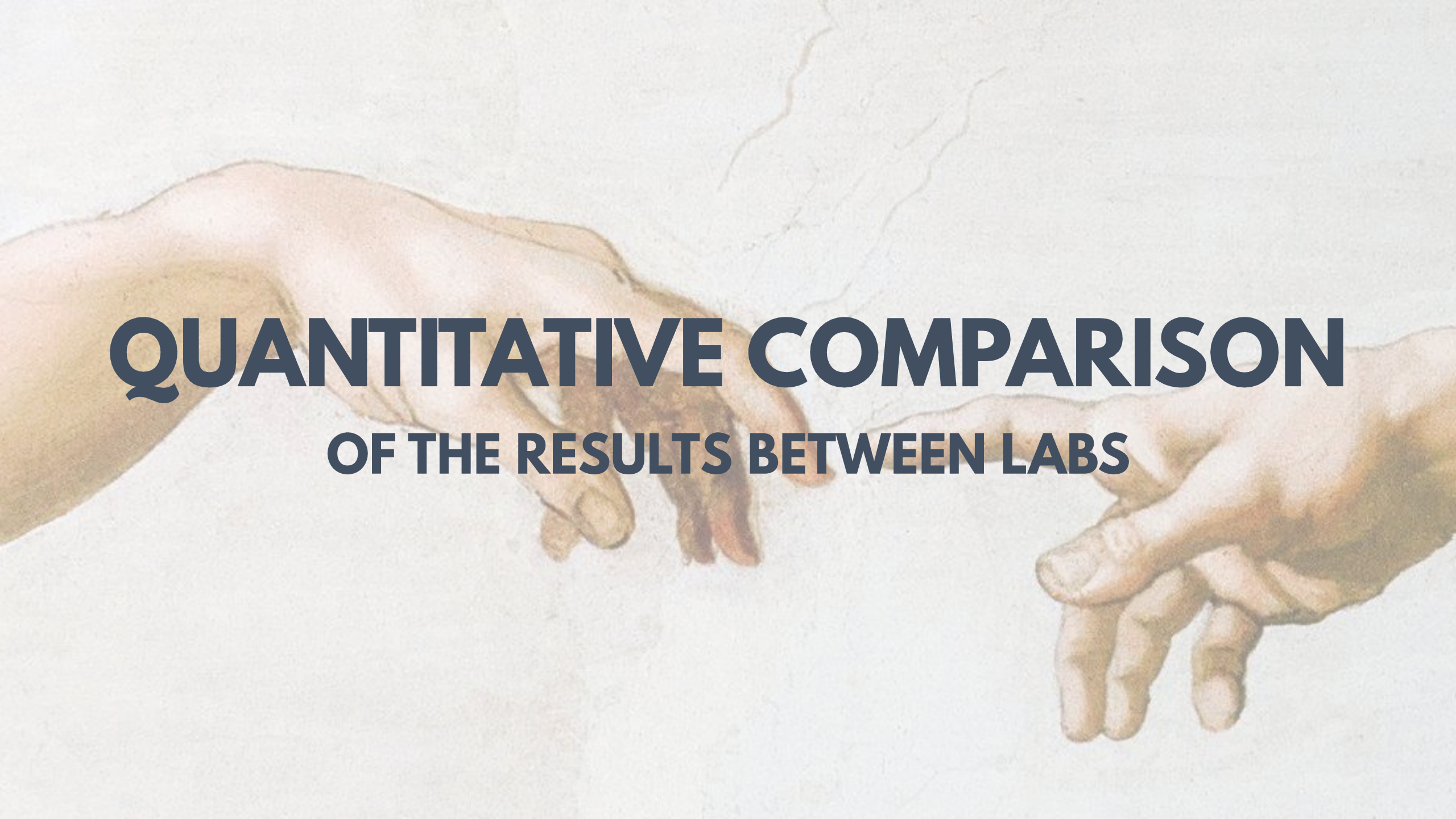
IN SURFACE WATER



28 contaminants from 28 surface water samples from the Netherlands

Quantified with targeted method

Mean prediction error **1.7x**



QUANTITATIVE COMPARISON

OF THE RESULTS BETWEEN LABS

INTERLABORATORY COMPARISON

134 PESTICIDES 6 CONCENTRATION LEVELS 6 CEREAL MATRICES

Testing Centre of University of Tartu

(Estonia)

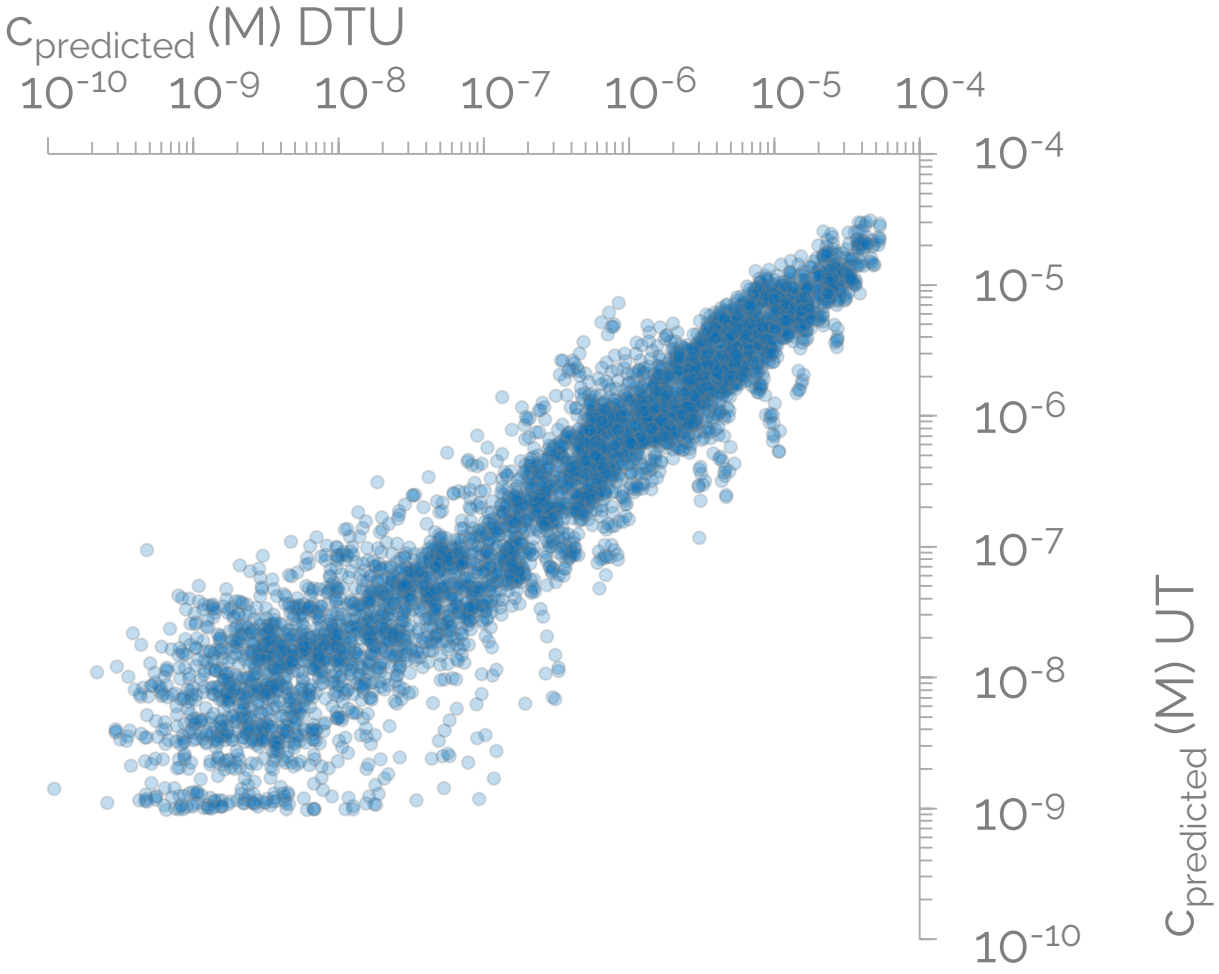
4863 pesticides identified

National Food Institute, Technical University of Denmark

4907 pesticides identified

COMPARABILITY BETWEEN LABS

CAN BE ACHIEVED WITH STANDARD SUBSTANCE FREE QUANTIFICATION



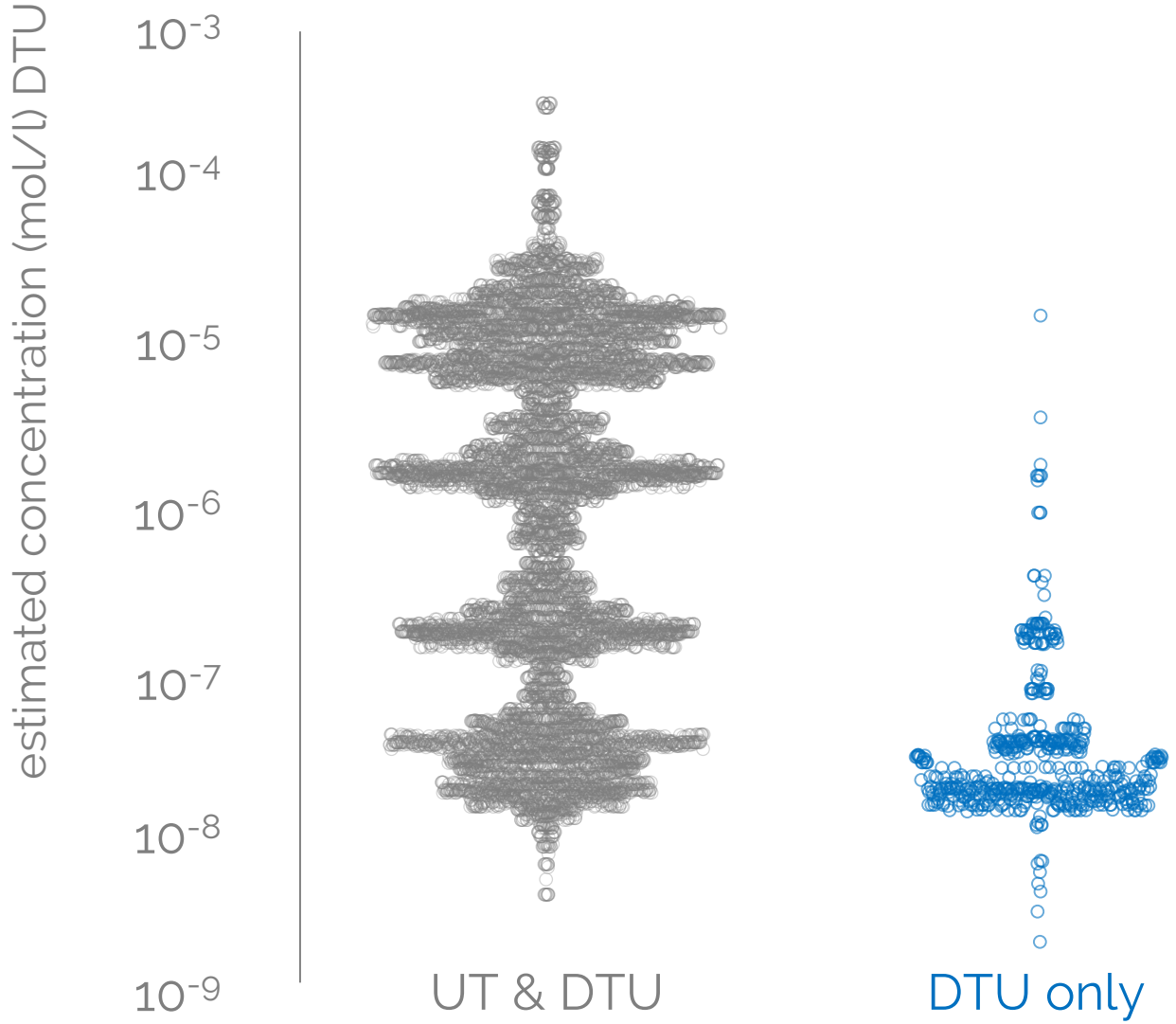
4856 data points

R^2 of 0.85
Average difference in concentration **3.2x**

89% of points with accuracy better than 10x

THE DIFFERENCES IN REPORTED PESTICIDES

RESULT PRIMARELY FROM DIFFERENT DETECTION LIMITS



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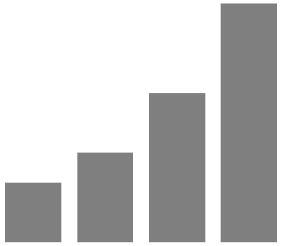
SUMMARY & FUTURE

OF THE COMPOUNDS DETECTED

FUTURE

FOR NON-TARGETED ANALYSIS

quantitative results

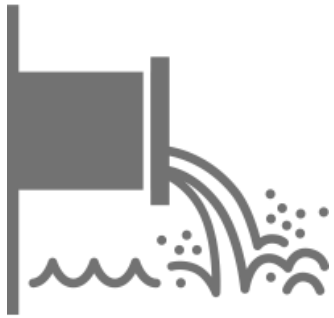


directly comparable data from different labs

retrospective analysis

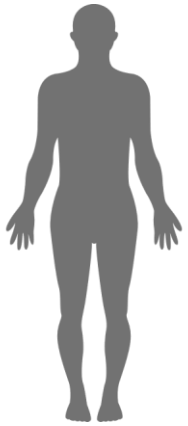
APPLICATION AREAS

environmental



food

metabolomics



pharmaceutical

OPEN POSITION

Post-doc in Computational Mass Spectrometry

OPEN POSITION

Full Professor in Analytical Chemistry in Stockholm University

Quantem ANALYTICS

quantem.co

Kruvelab.com

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